Towards Understanding *Pseudomonas aeruginosa* Infection through Global Expression Profiling: From Models to Real Infection Settings and Proposed Prevention Strategies

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I. Abbreviations

ANOSIM	Analysis of similarity
°C	Degree Celsius
CF	Cystic fibrosis
Cftr	Cystic fibrosis transmembrane conductance regulator
CFU	Colony forming units
CLO	Centre for Burn Treatment
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
e. g.	Exempli gratia (for example)
et. al.	Et alteri (and others)
FA	Formaldehyde agarose
g	Gram
h	Hour
l	Liter
M	Molar (mol/l)
m	Milli (10^{-3})
MDS	Multidimensional scaling
MHH	Medizinische Hochschule Hannover
min	Minute
mM	Millimolar
µ	micro (10^{-6})
n	nano (10^{-9})
no.	Number
OD	Optical density
PBS	Phosphate buffered saline
PCA	Principal component analysis
p. i.	Post infection
PQS	Pseudomonas Quinolone Signal; 2-Heptyl-3-hydroxy-4-quinolon
QS	Quorum sensing
RNA	Ribonucleic acid
rpm	Revolutions per minute
SIMPER	Similarity percentage of species contributions
SNP	Single Nucleotide Polymorphism
sec	Second
T3SS	Type III secretion system
TAE	Tris-acetate-EDTA
TE	Tris-EDTA
Tris	Tris(hydroxymethyl)-aminomethane
UV	Ultraviolet light
V	Volt
x g	Multiples of acceleration of gravity

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IV. Summary

Pseudomonas aeruginosa is a threatening opportunistic pathogen that causes severe acute and chronic nosocomial infections in immunocompromised and catheterised patients. It is prevalent in burn wound infections and is generally multi-drug resistant. Understanding the genetic programs underlying infection is essential to develop highly needed new strategies for prevention and therapy.

Global transcriptomic analysis of *P. aeruginosa* infecting various hosts was carried out. *In vivo* gene expression was successfully performed by developing accurate, specific technical procedures for sample collection, transportation and microarray preparation. The protocol was developed on burn wound infection samples and subsequently used for the analysis of CF patient sputum samples, as well as for lettuce and mouse tumour infection models. Gene expression of three multi-drug resistant *P. aeruginosa* burn wound isolates was profiled across all infection conditions and non-infection controls (planktonic and biofilm growth on rich LB medium). The transcriptomic analysis suggested that the main factors expressed by *P. aeruginosa* upon infection of burn wounds are iron and zinc acquisition as well as alginate production. The bacterial state during burn wound infection was not fully acute, with bacterial cells undergoing serious iron limitation and having a slower metabolism. Iron acquisition and alginate production were shown to be important mechanisms common among the infection conditions studied, namely burn wound, CF patient and mouse tumour model.

Two models for *P. aeruginosa* infection were tested. The tumour mouse model is a promising mammalian infection model whereby *P. aeruginosa* exhibits anaerobic growth, biofilm formation and expresses the type III secretion system. This model is being further tested in order to assess if it can be used as a chronic infection model. The plant infection model using lettuce leaves may be useful for the study of certain factors such as QS systems, but yielded different results as compared to the real mammalian infections and cannot therefore be used as a reliable infection model.

The multivariate statistical analysis of the combined expression data shows that the burn wound infection is most closely related in its global expression profile to the tumour infection model among all of the conditions. All tested infection and control conditions were statistically different from each other.

Finally, the Quorum Sensing (QS) inhibitory potential of a promising novel anti-pseudomonas compound, protoanemonin, was thoroughly tested. Its presence caused not only inhibition of QS but also induction of iron starvation regulated genes in *P. aeruginosa*.

The features observed and data generated here on *P. aeruginosa* upon infection of a host, provided a number of leads that can be considerably extended in future work. The effect of zinc and the regulation of zinc response may be a promising new path to understand and combat virulence of *P. aeruginosa*. In addition to the zinc effect and proposed targets such as glycine betaine production enzymes, there are a number of hypothetical unknown factors which may play a crucial role in infection. Examples are the proteins coded by the cluster PA4063-65 and PA4834-37, putatively coding for a novel siderophore system.

Summary

The analysis of the gene expression data from the work presented here thus provided a wealth of new insights and established a foundation for future work directed at the understanding of *P. aeruginosa* infection and at finding new prevention and treatment strategies.

1. Introduction

1.1. *Pseudomonas aeruginosa:* a dangerous opportunistic pathogen

Pseudomonas aeruginosa is a Gram-negative polar flagellated rod which is a member of the γ-proteobacteria group (Olsen *et al.*, 1994). It is widespread in nature and can be found inhabiting many environmental niches such as water, soil, plants and animals. *P. aeruginosa* is also an important opportunistic pathogen that can cause both acute infections in severe burns and urinary tract immunocompromised patients and also chronic infections in the lungs of patients with the genetic disease cystic fibrosis (Chugani & Greenberg, 2007). *P. aeruginosa* deriving from the patient's own endogenous gastrointestinal microbiota or from an environmental source are the most common causes of burn wound infections in many burn wound centres (Altoparlak *et al.*, 2004). Hospitals often harbour multi-resistant strains of *P. aeruginosa* which have been detected from hospital floors, bed rails, sinks and from the hands of medical personnel (Chitkara & Feierabend, 1981). Multi-drug resistant clones can remain in the hospital for many years because of transfer from patient to patient (Hsueh *et al.*, 1998) and the high percentage of patient mortality and morbidity render *P. aeruginosa* as one of the major nosocomial pathogens (Altoparlak *et al.*, 2004). Data collected over three years from the Centre for Burn Treatment in Siemianowice Śląskie (Poland) shows that both *P. aeruginosa* and *Staphylococcus aureus* are the major strains isolated from burn wounds (Table 1-1). Moreover, the antibiotic resistance of the clinical strains of *P. aeruginosa* is increasing with time (Table 1-2), which clearly complicates their eradication.

Diseases caused by *P. aeruginosa* are multifunctional and are connected with the production of several virulence factors (Lee *et al.*, 2006). Virulence factors and their role in infections have been studied for many years using a number of animal models. However, the correlation of the results obtained from the animal model generally diverged considerably from those of clinical infections (Rumbaugh *et al.*, 1999). In 1975, Stieritz and Holder developed a burn mouse model that was clinically relevant to burn wound sepsis. That

model was used to examine and identify a number of virulence factors and regulators that are now known to play an important role in burn wound infections (Saelinger *et al.*, 1977, Pavlovskis & Wretlind, 1979, Nicas & Iglewski, 1985, Arora *et al.*, 2005). These virulence factors can be differentiated into either cell associated factors such as adhesins, alginate, pili, flagella and lipopolysaccharides or extracellular factors such as elastase, exoenzyme S, exotoxin A, hemolysins, iron-binding proteins, leukocidins and proteases (Tredget *et al.*, 2004, Church *et al.*, 2006). It has only been more recently, with the development of medium and high-throughput gene expression techniques, that the dynamics and regulation of those virulence factors could be studied in the context of different infection models.

Table 1-1: Number of patients with nosocomial infections and percentage of pathogens isolated from burn wounds at the Centre for Burn Treatment, Siemianowice Śląskie, Poland (Bielecki *et al.* 2008).

Number of patient with	2003	2004	2005
Burn wounds	468	437	490
Nosocomial infections	207	135	191
Microorganism			
Pseudomonas aeruginosa	27	37	26
Staphylococcus aureus	27 (19 MRSA[a])	26 (13 MRSA[a])	30 (11 MRSA[a])
Acinetobacter baumannii	20	12	14
Proteus mirabilis	10	11	11
Escherichia coli	4	5	6
Klebsiella pneumoniae	5	5	4
Streptococcus pyogenes	3	2	5
Enterococcus faecalis	4	3	3
Candida spp.	0	1	0

a - *Staphylococcus aureus* MRSA- Methicillin-resistant *Staphylococcus aureus*

Table 1-2: Percentage of *P. aeruginosa* clinical strains resistant to given antibiotics. Strains were isolated between 2003 and 2005 in the Centre for Burn Treatment, Siemianowice Śląskie, Poland (Bielecki *et al.* 2008).

Antibiotic	2003	2004	2005
Colistin	0	2	0
Aztreonam	20	20	18
Tazocin	28	26	46
Meropenem	25	13	42
Imipenem	25	17	39
Ciprofloxacin	25	45	40
Ceftazidime	64	54	48
Amikacin	75	68	67

1.2. Burn wound infections

Thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humoral immune responses are pivotal factors contributing to infectious complications in patients with severe burns. The burn wound surface (in deep partial-thickness and in all full-thickness burns) is a protein-rich environment consisting of avascular necrotic tissue (eschar) that provides a favourable niche for microbial colonization and proliferation. The avascularity of the eschar results in impaired migration of host immune cells and restricts delivery of systemically administered antimicrobial agents to the area, while toxic substances released by eschar tissue impair local host immune responses (Church *et al*., 2006).

Burn eschar normally becomes colonized with the patient's own flora (predominantly Gram-positive bacteria) within 3-5 days of injury. This initial colonization is subsequently replaced by Gram-negative flora present in the hospital (Erol *et al.*, 2004), among which the *P. aeruginosa* is most common (Table 1-1). If the patient's host defences and therapeutic measures (including excision of necrotic tissue and wound closure) are inadequate or delayed, microbial invasion of viable tissue occurs, which is the hallmark of an invasive burn wound infection (Church *et al.,* 2006).

1.3. Cystic fibrosis pulmonary infections

P. aeruginosa pulmonary infection in cystic fibrosis (CF) patients is largely responsible for the high mortality rate (Lyczak *et al.*, 2000). CF is caused by mutations of the gene encoding the CF transmembrane regulator (CFTR), which functions as a chloride channel in epithelial membranes (Collins, 1992). The failure to secrete chloride by CFTR together with abnormal sodium absorption from the airway lumen results in isotonic salt concentrations, which increases mucus viscosity and impairs mucociliarity and clearance of the lungs (Ratjen & Doring, 2003). *P. aeruginosa* invades the mucus layer, which in deeper fractions is deprived of oxygen (Worlitzsch *et al.*, 2002). This makes the microenvironment of CF pulmonary infection different from that of the burn wound infection. Chronic infection with *P. aeruginosa* infection leads to epithelial surface damage and airway plugging, progressively impairing airway conductance which results in the decline of pulmonary function (Lyczak *et al.*, 2002).

1.4. Insights into pathogenecity through global gene expression profiling

The advent of sensitive molecular techniques has furthered our understanding of genomics through sequencing of the whole genome of relevant *P. aeruginosa* strains. The genome sequence and annotation of the strain *P. aeruginosa* PAO1, originally isolated from a burn wound in 1975 (Stieritz & Holder, 1975), was published in 2000 (Stover *et al.*, 2000). Then in 2006, Lee and colleagues reported the genome sequence of the highly virulent strain PA14. The sequencing of a further 5 strains is still in progress and can be monitored on the Pseudomonas Genome Database (www.pseudomonas.com). The availability of the whole genome sequences and annotations of these important strains open up new avenues to study infections. High-density DNA microarrays are a powerful tool to explore the complexity of *P. aeruginosa* genetic programs. Transcriptional profiling of *P. aeruginosa* allows us to gain insights into regulation networks and environmental responses underlying pathogenesis. Figure 1-1 presents the principles of microarray experiment.

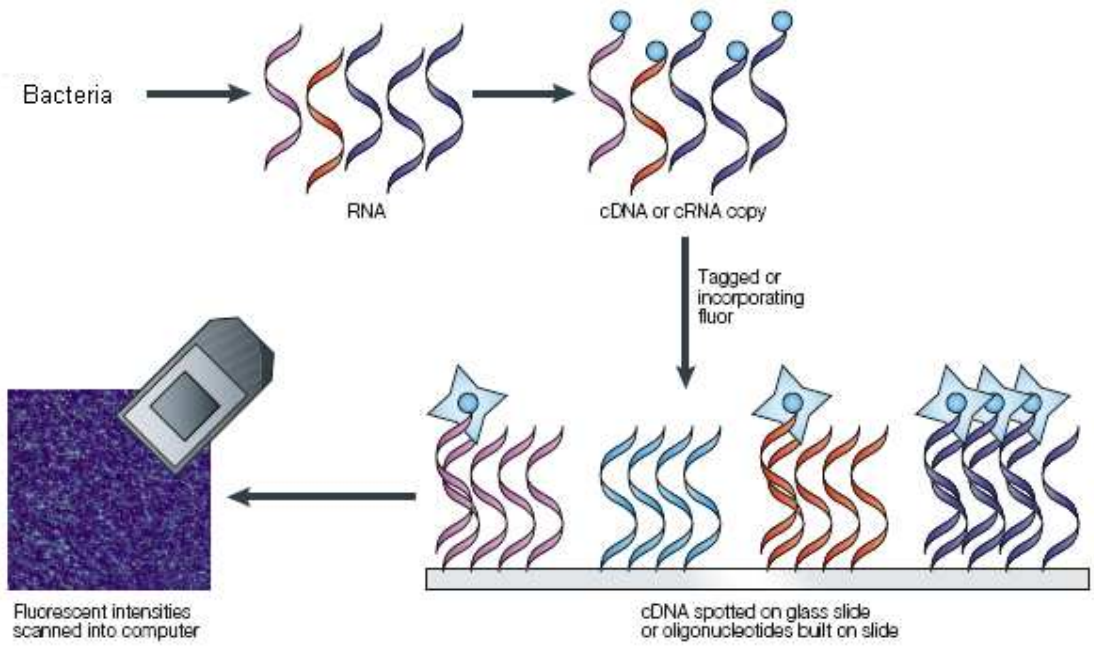


Figure 1-1: Experimental process of microarray preparation and analysis. Adapted from Butte (2002). RNA is isolated from bacteria, labelled and hybridised to the microarray. Then the array is scanned with laser light and the raw data analysed statistically.

With such techniques at hand, it has been possible to design and execute *in vitro* experiments under conditions known to be prevalent during host infection. Such conditions include: free-iron limitation in host tissues, oxidative stress generated by superoxide produced by polymorphonuclear leukocytes (PMNs) and biofilm formation, which both allows the pathogen to increase its resistance against antibiotics and to overcome nutrition shortage.

1.4.1. Iron acquisition

Bacteria require iron as a cofactor for redox-dependent enzymes. The ability to acquire iron ions from infected host cells is one of the important factors that enable the pathogen to thrive in the host and thus to trigger its virulence programs. During infection, iron is limited by the host as it is usually bound to proteins such as transferrin, lactoferrin, ferritin or bound as heme to hemoglobin. *P. aeruginosa* possesses a number of mechanisms based on iron-

chelating compounds called siderophores, such as pyoverdine or pyochelin as well as the heme uptake system (Takase *et al.*, 2000, Letoffe *et al.*, 1998). Pyoverdine acts also as a signal molecule that controls the production of secreted virulence factors like exotoxin A, endoprotease and pyoverdine itself (Lamont *et al.*, 2002) and as a signal for biofilm development (Banin *et al.*, 2005). Ochsner *et al* (2002) used the microarray technique to study bacterial responses to iron starvation while Palma *et al* (2003) examined the short-term response of iron addition to iron-starved *P. aeruginosa* cells. Furthermore, in addition to the genes previously known to be responsible for iron uptake and metabolism (namely pyoverdine, pyochelin and heme uptake) these studies found over 40 genes encoding for hypothetical and conserved hypothetical proteins. They also demonstrated that many regulators are iron-dependent. These studies underscore the role of iron in virulence and suggest obvious candidates for detailed functional analysis.

1.4.2. Oxidative stress response

Another type of stress that *P. aeruginosa* has to overcome upon infection, is the oxidative stress generated by the superoxide produced by PMNs, which is a host response to infection (Salunkhe *et al.*, 2005). Studies on the response to hydrogen peroxide were carried out in different experimental settings, such as the early adaptation response after exposing the cells for 10 and 20 minutes to hydrogen peroxide (Palma *et al.*, 2004, Chang *et al.*, 2005), or the steady-state response to hydrogen peroxide upon continuous exposure (Salunkhe *et al.,* 2005). These 3 studies on the effect of hydrogen peroxide revealed the activation of several protective mechanisms, including antioxidant defence systems such as catalase and DNA repair. Downregulation of the primary metabolism was also observed in these studies. Chang *et al* (2005) reported suppression of iron–uptake related genes as well as the induction of pyocins, which may be a defence mechanism against the oxidative attack by host cells. Both studies of Palma and Salunhke reported that iron regulated genes were activated upon exposure to hydrogen peroxide. However, only about 40% of the differentially regulated genes in the Chang experiment matched those in Palma's study. This was likely due to differences in the experimental design and procedures. Salunkhe and colleagues expanded

the experiment by using a reference strain of *P. aeruginosa* PAO1 and two strains isolated from the sputum of CF patients – TB and 892, which were two variants of the same clone. The transcriptional profiles indicated that strains named as TB and 892 were more proficient in their response to hydrogen peroxide (Salunkhe *et al.,* 2005). These findings illustrate the need for accurate experimental design of expression profiling and demonstrate that more than 1 clone should be examined in gene expression experiments to render conclusions more accurate.

1.4.3. Quorum sensing

Quorum sensing (QS) is the mechanism that allows bacteria to "sense" the density of the bacterial population and to respond to it in an organized manner, regulating thereby a large battery of genes, including those coding for virulence factors. The *P. aeruginosa* QS consists of three systems. Two of them are the directly interrelated *N*-acylhomoserine lactone (AHL)-dependent systems *las* and *rhl*. The third employs 2-heptyl-3-hydroxy-4-quinolone, the Pseudomonas Quinolone Signal (PQS) (Pesci *et al.*, 1999, Gallagher *et al.*, 2002, McKnight *et al.*, 2000, Diggle *et al.*, 2003). The complex interplay of these systems and the regulators involved is shown on Figure 1-2. The *las* system consists of the transcriptional regulatory protein LasR and its cognate signalling molecule, N-(3-oxododecanoyl) homoserine lactone (3O-C_{12}-HSL), whose production is directed by the autoinducer synthase encoded by *lasI.* The *rhl* system consists of the RhlR protein and an autoinducer synthase encoded by *rhlI*, which is involved in production of the cognate autoinducer N-butyryl homoserine lactone (C_4-HSL) (Smith & Iglewski, 2003, Wagner *et al.*, 2003). Although its production is enhanced by the *las* system, it can be formed in the absence of *lasR* (Diggle *et al.,* 2003). The PQS biosynthesis is regulated by the PqsR regulator, which was formerly known as MvfR. PqsR is a membrane-associated LysR-type transcriptional activator which also regulates elastase, phospholipase, 3-oxo-C12-HSL and pyocyanin (Diggle *et al.*, 2007a). Exogenous PQS induces expression of the elastase B genes and *rhlI* (McKnight *et al.,* 2000).

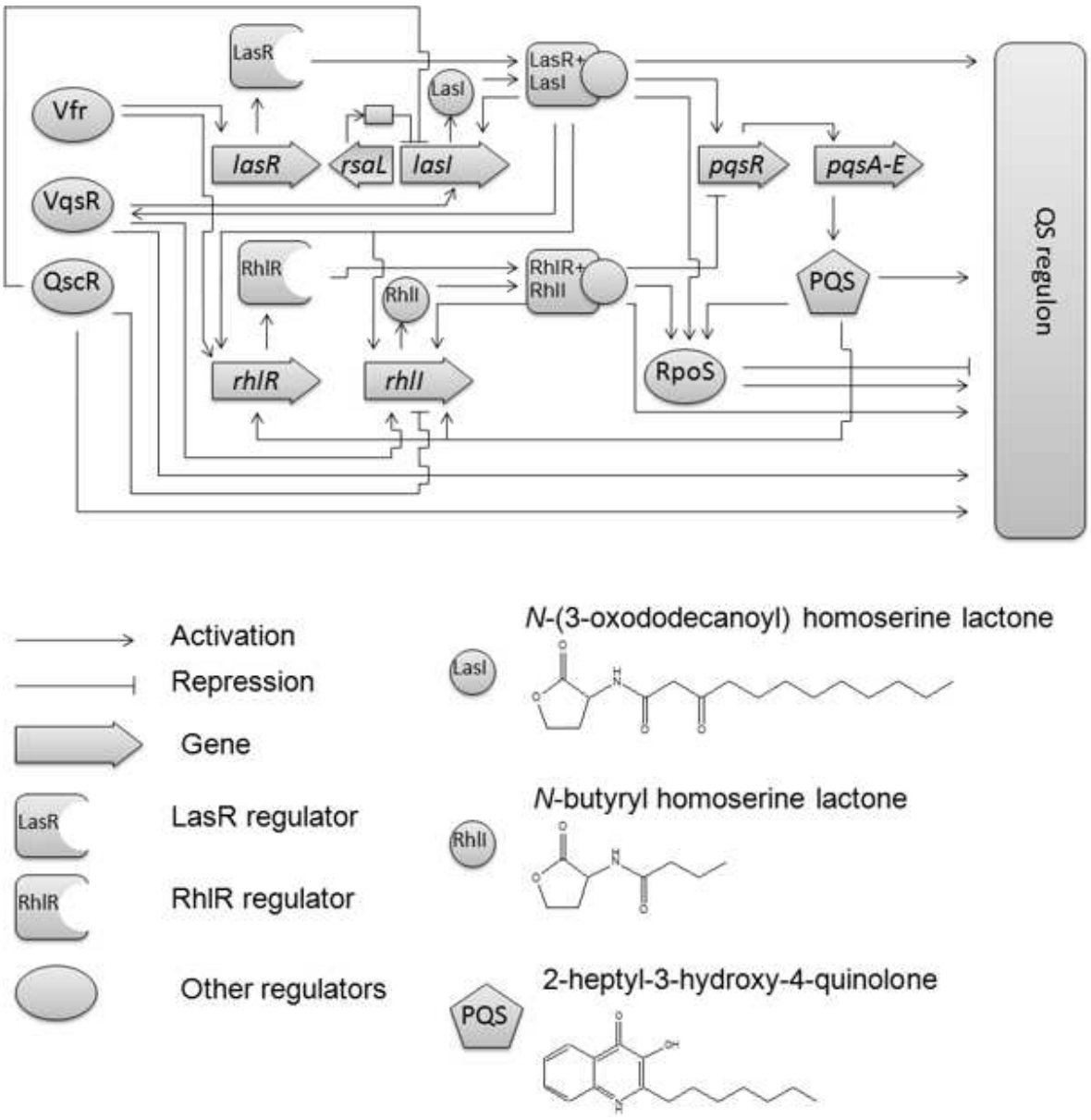


Figure 1-2: Quorum sensing systems in *P. aeruginosa.*

Rumbaugh and colleagues (1999) were the first to show the involvement of the QS systems in burn wound infections. They produced single *P. aeruginosa* mutants in *lasR*, *lasI* and *rhlI* as well as double *lasI* and *rhlI* mutants. In comparison to the *P. aeruginosa* parent strain PAO1, *lasI* and *rhlI* mutants were significantly less virulent. In addition, both mutants were much less efficient than PAO1 in spreading across the bodies of burnt and infected mice. The most significant decrease in virulence was observed in the mutant with both *lasI* and *rhlI*

disrupted, which pinpoints the combinatorial influence of genes and interacting traits in virulence and, as referred to above, is the hallmark of *P. aeruginosa* pathogenesis (Lee *et al.,* 2006). It was also discovered that the strain with a disrupted regulatory gene *lasR*, which was incapable of producing elastases (LasA and LasB), exotoxin A and alkaline protease, was also unable to disseminate to distal host sites in a colonized burn wound. The virulence of strains with the non-disrupted *lasR* gene but with specific mutations in *lasA*, *lasB*, *toxA* (encoding exotoxin A) and *rpoS* gene was not as strongly reduced as in the strain with the *lasI* and *rhlI* mutation. Moreover, all these mutants were able to spread effectively across the burned skin and the body of the infected mice (Rumbaugh *et al.*, 2000). This fact suggests that QS regulates other virulence factors that are important in burn wound infections which were not known at that time, and that overall virulence is mediated by a larger set of these factors. Its full complexity has only been revealed once genome-scale expression profiling became available.

Hentzer *et al.,* (2003), Schuster *et al.,* (2003) and Wagner *et al.,* (2003) examined the global QS response to the effect of different purified acyl-homoserine lactones on autoinducer synthase mutants. The joint analysis of the data from these experiments provides a comprehensive overview of the QS regulon, that is, the joint set of genes that are regulated by QS systems. In addition, there are sets of data from experiments made on other regulators, which together, co-regulate the *las* and *rhl* QS circuits. These include: i) Vfr – a global regulator of *P. aeruginosa* virulence, which responds to the alarmone cAMP and induces *lasR* transcription (Albus *et al.*, 1997, Wolfgang *et al.*, 2003); ii) QscR – LuxR type regulator without cognate synthase. It delays the activation of several QS controlled genes and activates its own set of target genes (Lequette *et al.*, 2006) iii) VqsR – a virulence and quorum sensing regulator (Juhas *et al.*, 2005); iv) the alternative sigma factor RpoS (Schuster *et al.*, 2004); v) PrpB – a two component response regulator (Dong *et al.*, 2005); and vi) MvfR (PqsR) – a multiple virulence factor regulator that activates the direct precursor of PQS, as referred to above (Deziel *et al.*, 2005). Recently, Bredenbruch *et al* (2006) reported that, in addition to its known function as a signal molecule, PQS has iron-chelating activity in the cell, leading to activation of iron acquisition and oxidative-stress response genes. It has also been

suggested that the PQS's location in the cell envelope may facilitate siderophore-mediated delivery, although PQS itself does not function as a siderophore (Diggle *et al.*, 2007b). Furthermore, it was shown that the PQS precursor 2-heptyl-4(1*H*)-quinolone (HHQ), which does not form an iron complex, can act as a signal molecule *per se* (Diggle *et al.,* 2007b).

1.4.4. Biofilm formation

The ability to form biofilms is one of the essential factors for the prevalence of infection. Biofilm formation provides protection to bacteria from a wide range of environmental challenges, such as UV exposure, metal toxicity, acid exposure, dehydration and salinity as well as infection challenges such as phagocytosis and several antibiotics and antimicrobial agents (Hall-Stoodley *et al.,* 2004). The crucial role of biofilms in *P. aeruginosa* chronic infections such as cystic fibrosis and otitis media have been well established (Singh *et al.*, 2000, Ehrlich *et al.*, 2002). Recently Schaber and colleagues examined the role of biofilm in a thermally injured mouse model of acute infection (Schaber *et al.*, 2007). Using light, electron and confocal scanning laser microscopy they demonstrated that *P. aeruginosa* forms biofilms in burn wounds after 8h of infection and bacterial cells congregate around the blood vessels. Importantly, the same study showed that a QS mutant strain PAO1-JP2, which is known to be less virulent in burn wound infection, was still able to form biofilms in burn wounds. The authors suggested that QS is needed for efficient blood vessel invasion subsequent to biofilm formation and following the increase in local cell density (Schaber *et al.,* 2007). Several studies on biofilm development by *P. aeruginosa* were designed as a comparison of planktonic cultures with those at different stages of biofilm formation (Hentzer *et al.*, 2005, Waite *et al.*, 2006, Waite *et al.*, 2005). These studies revealed that the profiles of the genes expressed at the different stages of biofilm development were similar to those of the planktonic stationary phase, which underscores the hypothesis that the majority of the bacteria present in a biofilm are in stationary phase physical state. Biofilm formation involves several adaptive responses such as anaerobic oxidation and iron-limitation stress (Hentzer *et al.,* 2005).

Another study measured the genome-wide transcriptional response of a biofilm exposed to the beta-lactam antibiotic imipenem (Bagge *et al.*, 2004). The *P. aeruginosa* biofilm induced by addition of imipenem revealed more than five-fold differential regulation of 34 genes as compared to the non-induced biofilm control. The most upregulated gene was the *ampC,* which encodes for a beta-lactamase precursor. Also, the alginate biosynthesis pathway was upregulated, whereas the flagellum-encoding and pilus-encoding genes were downregulated in the imipenem-exposed biofilm (Bagge *et al.,* 2004). The expression of the alginate pathway is dependent upon the sigma factor AlgU. AlgU also induces the transcription of *rpoH*, which regulates different stress responses. Moreover, AlgU has been associated with the negative control of flagellum synthesis (Bagge *et al.,* 2004). Although alginate itself is not a barrier for beta-lactam antibiotics, the high numbers of beta-lactamases produced by *P. aeruginosa* may accumulate in the extracellular biofilm matrix and hydrolyse beta-lactam antibiotics as they penetrate the biofilm (Bagge *et al.,* 2004). QS systems have been suggested to be essential for biofilm formation and have been reported to be involved in biofilm-specific gene expression (Davies *et al.*, 1998, De Kievit *et al.*, 2001). However, this statement was later challenged by experiments in which QS mutant strains were able to form similar biofilms to the parent strains (Heydorn *et al.*, 2002, Schaber *et al.,* 2007). Hentzer and colleagues tested the QS regulon in biofilms and found that about 80% of the previously reported QS regulated genes were differentially expressed. Interestingly, 144 genes were QS regulated specifically in biofilm growing cells. Forty-four percent of the genes from this biofilm-specific, quorum-sensing regulon were related to the response to iron limitation, as reported previously by Ochsner (2002). These findings pinpoint an important link between QS and the iron regulatory system (Hentzer *et al.,* 2005).

Altogether, the various experiments described above provided us with a relatively extensive list of genes differentially regulated under the various conditions. This “library” can be used as a reference for all subsequent experiments and may complement them, although it should be emphasized that the impact of differences in experimental settings, such as growth phase, physical and chemical conditions may be the reason for the relatively poor correlation among the lists of genes generated in those various studies.

Despite its intrinsic value, a list of differentially regulated genes *per se* remains simply a list, a catalogue of relatively dispersed information. The real challenge lies on understanding how these gene sets are embedded in the various hierarchical metabolic and regulatory networks operating in *P. aeruginosa,* and on how these networks behave as a function of changing environmental conditions and interactions with the host. Clearly, it is this wiring and complex interplay of the many regulatory and metabolic networks that renders *P. aeruginosa* highly effective in its infection and persistence.

1.4.5. Host-pathogen interactions

Although the *in vitro* experiments described above yielded important insights into some of the factors involved in virulence, they remain laboratory experiments with only indirect relation to the real infection and host-pathogen interactions. More recently, experiments have involved placing *P. aeruginosa* in contact with eukaryotic cells and monitoring its global transcriptional response. Such approaches attempt to mimic more realistic infections by *P. aeruginosa.* Several models were developed to mimic lung infection in cystic fibrosis patients. Frisk *et al.* (2004) measured the transcriptional profiles of *P. aeruginosa* following interaction with primary normal human airway epithelial cells (PNHAE) after 4 and 12 hours. After 4 hours, 41 genes were differentially expressed with regard to a control consisting of *P. aeruginosa* cells growing in the same medium but without the presence of eukaryotic cells. Among the activated genes, there were 24 encoding putative proteins involved in membrane transport as well as 3 encoding transcriptional regulators. Interestingly, the pyoverdine pathway involved in iron acquisition was downregulated as well as the gene *pchD* encoding for a pyochelin biosynthesis protein. After 12 hours, 121 genes were differentially regulated. Several genes involved in phosphate acquisition were significantly upregulated, as well as the virulence factor *plcN* encoding a nonhemolytic phospholipase C precursor, which hydrolyzes phosphatidylcholine and phosphatidylserine present in both the outer and inner leaflets of eukaryotic erythrocytes, respectively. A possible role for PlcN in *P. aeruginosa in vivo* lung infection may be relevant since phosphatidylcholine is also an abundant

constituent of the lung surfactant and thus may serve as a substrate for the extracellular enzyme PlcN (Konig *et al.*, 1997, Frisk *et al.,* 2004).

The genes responsible for siderophore-mediated iron acquisition were downregulated at both 4 and 12h following contact with eukaryotic cells. Since the pyoverdine, pyochelin and iron-regulated genes have been earlier shown to be important for *P. aeruginosa* infections (Vasil & Ochsner, 1999, Lamont *et al.,* 2002) these results were surprising. The authors suggested that iron might be released upon damage of PNHAE cells caused by interaction with *P. aeruginosa* cells (Frisk *et al.,* 2004). They also suggested that a non-siderophore-mediated iron acquisition system, such as heme uptake may play an important role in *P. aerugionosa* infections (Takase *et al.,* 2000).

Another interesting experiment performed with the epithelial cells involved using the wild-type strain of *P. aeruginosa* PAO1 and a mutant unable to synthesise the type III secretion system and rhamnolipids. The infection with the mutant resembled a chronic infection, where there was no significant damage to the epithelial cells, whereas the wild-type strain mimicked the acute infection with injury to the epithelium (Chugani & Greenberg, 2007). Both strains were grown on primary different human airway epithelia and in TSB medium as the control. All data sets were compared and the differentially expressed genes were grouped into overlapping or specific genes to chronic and acute infection. In the overlapping set, 82% and 74% of the genes for the wild-type and mutant, respectively, had been previously shown to be activated by iron starvation (Ochsner *et al.,* 2002). This finding is on the contrary to Frisk *et al.,* but since there is considerable evidence that bacteria infecting the host have to overcome the low-iron availability (Schaible & Kaufmann, 2004) and taking into account that Chugani and Greenberg were able to prevent injury to the epithelial cells in their experiments, they assumed that this miscorrelation was simply due to the different experimental settings which again highlights the need for standardization in experimental design.

Another approach to understand pathogenesis of lung infections in cystic fibrosis patients was taken by Wolfgang *et al.* 2004, who profiled global gene expression in response to

muco-purulent respiratory liquid derived from chronically infected adult CF patients. This work reported the activation of the Rhl-dependent quorum sensing network and repression of a number of genes encoding proteins involved in flagellar-mediated chemotactic motility including *fliC*, which encodes for flagellin (Wolfgang *et al.,* 2004). Flagella are highly immunogenic, so that downregulation of *fliC* is likely to be an adaptive response to avoid host defences. Interestingly, the authors also compared their microarray data with the data sets of QS regulated proteins by Schuster *et al.* (2003) and found that a number of genes, not previously reported to be part of the QS regulon, were significantly activated or repressed in the context of CF respiratory liquid response (Wolfgang *et al.,* 2004). While in their experiment the putative operon encoding a complete ABC transport system (PA2327-PA2331) was repressed by the QS system, this system had been reported to be activated by QS in the study by Schuster *et al* (2003). Wolfgang *et al* (2004) suggested that QS signalling in the lung environment extends beyond the response network observed in laboratory settings (Wolfgang *et al.,* 2004). This important and unsurprising observation is crucial for the design and execution of future *in vivo* experiments and for the expectations that may be derived from them.

1.5. *In vivo* infection models

P. aeruginosa is a classical opportunistic pathogen and is able to cause infections in a range of higher organisms. The range of host species has an advantage for research in permitting the development of numerous infection models (Williams *et al.*, 2007). Adding to the well established murine models (Stieritz & Holder, 1975) we can also add a range of non-vertebrate eukaryotic infection models. These models include *Caenorhabditis elegans*, *Drosophila melanogaster*, *Dictyostelium discoideum* (a soil-leaving amoeba), *Arabidopsis thaliana*, *Galleria mellonella* (greater wax moth), silkworm larvae, alfalfa and lettuce (Rahme *et al.*, 1997, Tan & Ausubel, 2000, D'Argenio *et al.*, 2001, Cosson *et al.*, 2002, Kaito *et al.*, 2002, Silo-Suh *et al.*, 2002, Miyata *et al.*, 2003).

The considerable differences reported among similar *in vitro* experiments that stemmed, unto a large extent, from the variability in experimental settings (Vasil, 2003), clearly show

that the true picture of bacterial infection and host-pathogen interactions can be achieved only by means of thorough *in vivo* experiments. So far, only a few laboratories have succeeded in performing global gene expression profiling of pathogens at the site of an infection. This is largely due to some technical obstacles such as RNA isolation, insufficient amount of mRNA and difficulties with finding a realistic *in vivo* model. For the burn wound to date there is no published work on the *in vivo* gene expression program of *P. aeruginosa.* A very interesting *in vivo* experiment was performed by Mashburn *et al.* (2005) by growing *P aeruginosa* in rat peritoneum. A *P. aeruginosa* strain was inoculated in a dialysis membrane chamber (DMC) and implanted into the peritoneal cavity of rats. Two independent experiments were carried out in this setting. In the first, *P. aeruginosa* was cultured alone, whereas in the second, *P. aeruginosa* grew in a co-culture with *Staphylococcus aureus*. The latter experiment mimicked well a real infection as during infection of either CF or burn wound patients, *P. aeruginosa* is rarely present alone and it tends to appear in combined infections (Hoffman *et al.*, 2006). Mashburn and colleagues were able to perform microarray analysis of *P. aeruginosa* in co-culture with S*. aureus* because of differences in RNA extraction methods of the Gram negative and the Gram positive bacteria, which enabled them to separate the *P. aeruginosa* RNA. It is currently very difficult to separate the RNA from different Gram negative bacteria.

As in previous studies, iron acquisition systems were shown to play a crucial role in the homogeneous *P. aeruginosa* experiments. Genes involved in synthesis and binding of pyoverdine and pyochelin were induced as well as genes involved in heme uptake, such as the gene *hasAp,* which was induced over 2000-fold. The induction of genes encoding proteins involved in transport and metabolism of amino acids indicates that *P. aeruginosa* uses amino acids as a carbon source in the peritoneum (Mashburn *et al.,* 2005). Also two of the previously isolated genes by IVET, *fptA* and *np20,* were significantly upregulated in the pure-culture setting. In the *in vivo* co-culture experiment with *S. aureus*, the most striking feature as compared to that with *P. aeruginosa* alone was that 95% of repressed genes during growth in the presence of *S. aureus* were regulated by iron availability (Mashburn *et*

al., 2005). To explain this, the authors proposed a model where *P. aeruginosa* lyses the *S. aeureus* and uses the released iron to grow under a low-iron environment.

In vivo transcriptional profiling of the samples collected from infected patients will certainly provide invaluable insights into host-pathogen interactions and into the mechanisms of pathogenesis. This is one of the main aims of this PhD work. So far, there are still only few such experiments reported for microbial infections in humans. In 2005, LaRocque *et al.* reported on the *in vivo* expression profiling of *Vibrio cholerae* during early and late infection, and Roos and Klemm (2006) described a experiment with *Escherichia coli* in the context of human urinary tract infection. In both studies the authors compared and analysed the differences between the differentially regulated genes obtained from their studies and those from *in vitro* growth from previous experiments. Unsurprisingly, these analyses showed that *in vivo* programs are much more complex than those obtained in *in vitro* experiments due to the influence of many and possibly still unknown factors. However, for *P. aeruginosa*, it was not until more recently that a study reported transcriptomic profiling of *P. aeruginosa*'*s* mRNA extracted directly from lungs of a Cystic Fibrosis patient (Son *et al.*, 2007). A highlight from this experiment was the up-regulation of the genes responsible for antibiotic resistance and for the use of nutrient sources used by *P. aeruginosa* in the lung, which comprise: lung surfactant and amino acids. The same study compared the expression patterns from a clinical strain and a PAO1 type strain. They found that, in contrast to the PAO1 control, many virulence factors including alginate, lipase, phospholipase, rhamnolipid hemolysin, hydrogen cyanide production and pyochelin, are constantly expressed in the clinical strain. Although the study involved only one patient, the results provide evidence for the adaptation of *P. aeruginosa* strain to the CF lung environment over years of chronic infection, which is consistent with biomedical and epidemiological observations in various clinical studies (Smith *et al.*, 2006)

1.6. New anti-pseudomonal targets

One of the pivotal targets for treatment of *P. aeruginosa* is the QS sensing system, for which there are a number of inhibitors (QSI) that act upon the cells. Briefly, there are three

possibilities to target and inhibit QS: i) by blocking AHL production, ii) by inactivating signal molecules and, iii) by jamming the signal receptor. The latter is the most extensively studied in the context of drug development. Rasmussen and colleagues (2005a) screened a vast library of synthetic and natural compounds to test if they can act as QSI. The most active synthetic compound was 4-nitro-pyridine-*N*-oxide (4-NPO) while among the natural compounds a garlic extract was the most inhibitory. Transcriptomic analysis confirmed that QS regulated genes were inhibited by both blockers. Furthermore, the study found that both compounds reduce the tolerance of the *P. aeruginosa* biofilm to tobramycin and virulence in a *Caenorhabditis elegans* infection model (Rasmussen *et al.,* 2005a). Garlic was also shown to promote rapid clearing of *P. aeruginosa* from the lungs in QSI-treated mice in a pulmonary infection model (Bjarnsholt *et al.*, 2005). In addition, penicilic acid and patulin, produced by *Penicillium* species, were shown to target 45% and 60% of the *P. aeruginosa*'s QS regulated genes, respectively (2005b). However, the most widely studied group of compounds is that of halogenated furanones, which have been reported to interfere with the QS systems (Givskov *et al.*, 1996, Manefield *et al.*, 2002). Furanone C-30, a synthetic furanone derivative, was found to inhibit almost 80% of the QS regulated genes and also to attenuate *P. aeruginosa* in a pulmonary mouse model (Hentzer *et al.,* 2003). The biofilm formed in the presence of furanone C-30 is susceptible to grazing by PMN (Rasmussen *et al.,* 2005a, Bjarnsholt *et al.,* 2005). Another potential compound of interest is the macrolid azithromycin, which is conventionally used against gram-positive bacteria. Recently, the impact of this drug was assessed at the transcription and protein expression level of *P. aeruginosa* under infection-related conditions. Azithromycin inhibited the QS regulon as well as formation of biofilm (Nalca *et al.*, 2006), and it was shown to affect the polymerisation of alginate (Hoffmann *et al.*, 2007). Its QS inhibitory properties were also confirmed *in vivo* in a CF mouse model (Hoffmann *et al.,* 2007).

Most QS inhibitory compounds proposed so far have been targeted at Cystic Fibrosis patients. The first steps in bacterial burn wound infections are the colonisation of the wound and subsequent formation of biofilms on the epithelial surface. However, although QS systems are undoubtfully important in the invasion of blood vessels by *P. aeruginosa*

(Rumbaugh *et al.,* 1999*,* Schaber *et al.,* 2007), colonisation and biofilm development may be achieved independently of QS systems, as demonstrated by Schaber *et al.* (2007). Even when bacteremia from burn wound infection is not developed, the colonisation itself also complicates the treatment of burn patients. Infected wounds cannot be treated with skin grafts and persistent burn wound infection greatly extends the time of hospitalisation. One possibility is to introduce siderophore-like antibiotics, which may be transported by the bacterial cell, which recognizes the antibiotic as its own siderophore (Budzikiewicz, 2001, Braun & Braun, 2002). Recently, the synthesis of such compounds, namely pyochelin–norfloxacin conjugates was reported (Rivault *et al.*, 2007). Further research is needed in order to assess the bactericidal properties on different *P. aeruginosa* isolates. Another approach to target iron acquisition was developed by Furci *et al.* (2007), who proposed the inhibition of bacterial heme oxygenase, which is responsible for the last step in heme utilisation. By screening *in-silico* inhibitory compounds against certain 3D enzyme structures and through subsequent experimental assays, the group was able to identify novel inhibitors of the heme oxygenase of *Neisseria meningitidis*, which were also active against *P. aeruginosa*'s heme oxygenase but not against the human kind which is an encouraging result. Another line of investigation is the blockage of siderophore synthesis. In this regard, Ferreras *et al.* (2005) reported the synthesis and evaluation of a small molecule, salicyl-AMS, which inhibits the synthesis of salicylic acid-derived siderophores like mycobactins from *Mycobacterium tuberculosis*, yersiniabactin from *Yersinia pestis* and importantly pyochelin from *P. aeruginosa*. Thus, iron acquisition systems are becoming one of the most promising drug targets (Ferreras *et al.,* 2005, Quadri & Luis, 2007). Clearly, much more research is necessary for the development of functional antimicrobial agents effective against multi-resistant infections. Genome-wide and targeted expression profiling will be central in this endeavour.

1.7. Analysis of transcriptomic data

Microarray technologies allow researchers to determine expression of several thousands of genes simultaneously. The Affymetrix *P. aeruginosa* GeneChip® contains probe sets

representing 5900 *P. aeruginosa* gene transcripts and various controls. Once the raw data from hybridisation are obtained, they are subjected to thorough analysis. It starts with data normalisation and the quantification of gene expression levels. The approaches used to analyse the gene expression data set can be divided into two categories: supervised and unsupervised. Supervised routines are used for finding the expression levels that are significantly different between groups and finding those genes that accurately predict a characteristic of the sample (Butte, 2002). Unsupervised routines seek to find internal structure or relationships in the global data set. Figure 1-3 presents the scheme that could be used to analyse microarray data sets. A detailed review about each routine is beyond the scope of this introduction and can be found elsewhere (Brazma *et al.*, 2001, Knudsen, 2002, Amaratunga & Cabrera, 2004). There is a number of methods used for filtering differential expression such as fold change or rank products (Breitling *et al.*, 2004). Under the umbrella of unsupervised approaches, the most often used routines are: cluster determination for determining groups of genes or samples with a similar pattern of gene expression using hierarchical clustering or self-organizing maps (Golub *et al.*, 1999, Tamayo *et al.*, 1999); feature determination and data visualisation of genes or conditions using ordination approaches such as principal component analysis (PCA) (Alter *et al.*, 2000) or multidimensional scaling (MDS) (Tzeng *et al.*, 2008).

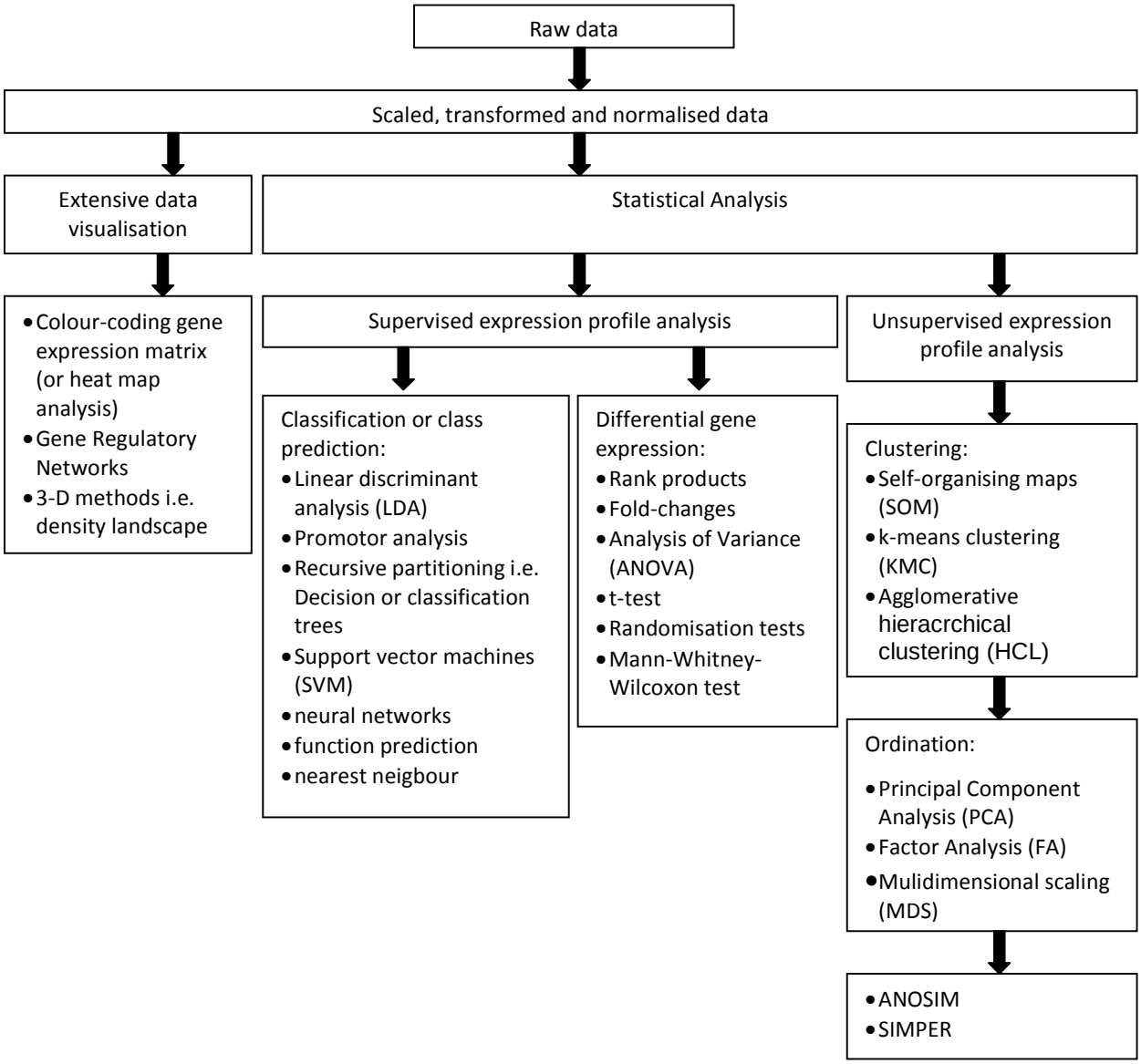


Figure 1-3: Scheme of the typical microarray data analysis approach using supervised and unsupervised routines.

2. Project rationale

With the increasing prevalence of bacterial infections across the world, a deeper understanding of the pathogen behaviour upon the infection is fundamental for the development of effective treatment strategies. Global *in vivo* gene expression profiling is a powerful tool to gain insights into the pathogen behaviour upon infections. It may also provide the basis for the discovery of novel virulence systems or rediscovery of factors that had not been previously considered important for infection. The scope of this work was thus to evaluate the genetic expression programs used by *P. aeruginosa* upon real *in vivo* infection conditions. The main focus was on burn wound infections and their comparison with other infections and infection models.

The approach followed was to compare the gene expression of *P. aeruginosa* infecting burn wounds and CF patients with different models that attempted to capture the effects of infection. To that end, a number of technical aspects had to be addressed, namely, the development of an accurate method to collect the material (RNA) and to technically obtain gene expression profiles from *P. aeruginosa* clones from an *in vivo* infection. The availability of realistic infection models is crucial for the testing of the hypotheses generated. Two models were proposed: a lettuce infection model and a novel mouse tumour infection model. These two models were assessed thoroughly by the analysis of global expression patterns.

Finally, the effects of a potentially novel anti-pseudomonal compound, protoanemonin, were tested using a combined transcriptomic and proteomic approach, coupled with QS inhibition assay.

Thus, by drawing on the current knowledge on *P. aeruginosa* infections, this PhD thesis was designed to answer a number of questions related to the following aims:

i) To elucidate the genetic programs used by *P. aeruginosa* during different infections:

- What are the main factors expressed by *P. aeruginosa* during a burn wound infection?
- What are the genetic programs used by *P. aeruginosa* in pulmonary infection of cystic fibrosis?
- Which factors are common to both infections and which are condition-specific?

ii. To appraise various models mimicking *in vivo* infections:

- Are there any good infection models that can mimic real *in vivo* infections?
- Is the lettuce model adequate and can plant infections be used to compare the transcription profile under different mammalian infectious conditions?
- Is our novel mouse tumour model colonised by *P. aeruginosa* a good chronic infection model?
- How does the transcription profiling compare among all the different conditions?
    - Can we find any trends?
    - Are there commonalities between different infection sites and models?
- Does a global transcriptomic study provide us leads and the means to find possible targets for infection prevention?

iii. To test the potential of an anti-pseudomonal compound – protoanemonin as a case study:

- What is the impact of protoanemonin on the level of global expression?
- What is the response of *P. aeruginosa* measured by secreted proteins?
- Can protoanemonin be proposed as novel anti-pseudomonal compound?

3. Materials and Methods

The general overview of the transcription experiments is presented on Figure 3-1 with indication of sections, in which given techniques or protocols are described. Other Materials and Methods are described within the chapter.

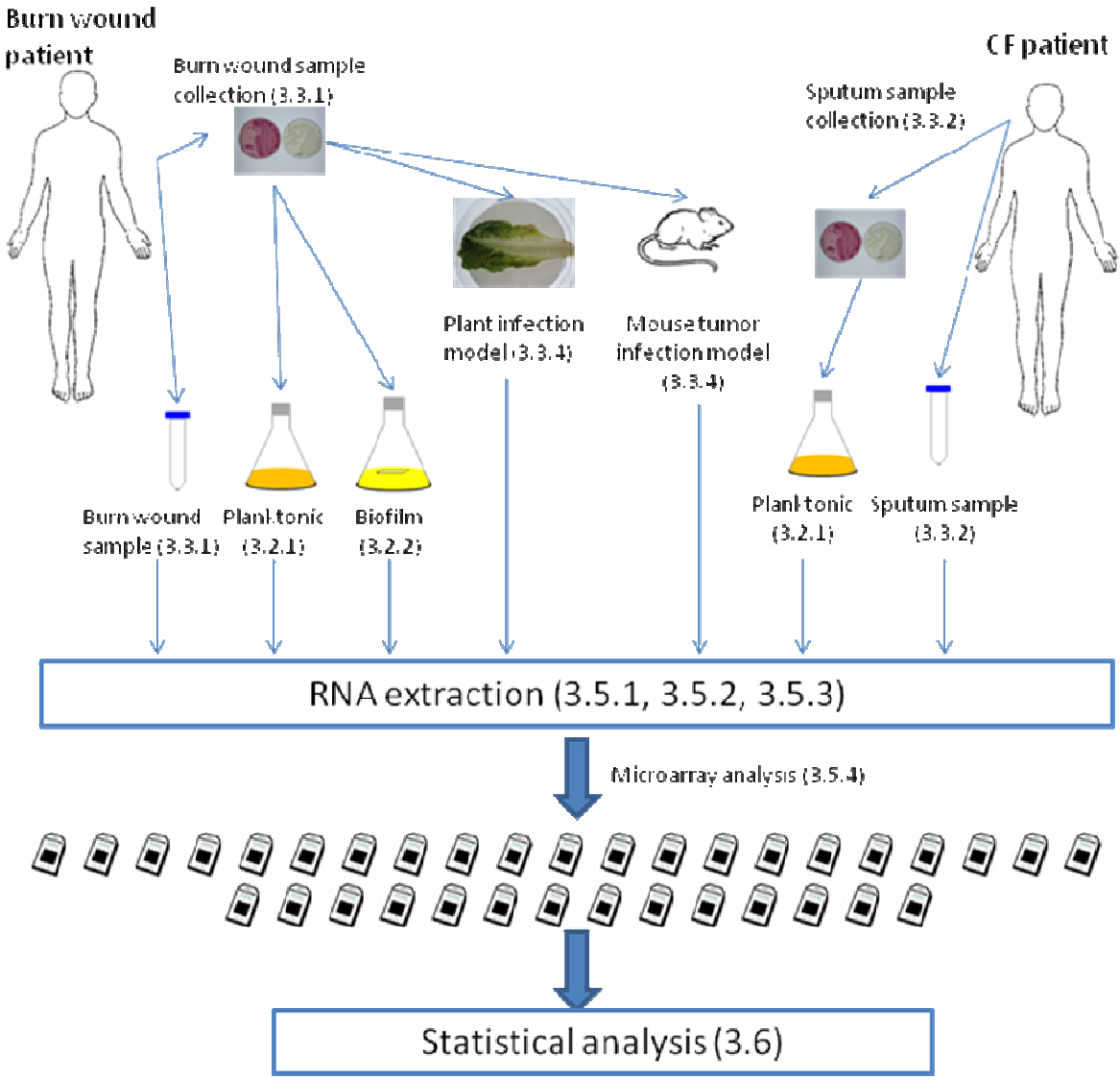


Figure 3-1: An overview of the experimental approach used to address the aims of this thesis, highlighting the key techniques used to perform global transcript analysis of *P. aeruginosa*. The numbers shown in parentheses indicate the section where the protocols are described in full.

3.1. Strains and Media

3.1.1. Bacterial Strains

All bacterial strains used in this work are listed in Table 3-1.

Table 3-1: Bacterial strains used in this work

Bacterial strain	Description	Origin
Escherichia coli TOP10	Electrocompetent cells	Invitrogen
Escherichia coli HB101(RK600)	Conjugation helper strain	Boyer and Roulland-Dussoix, 1969
Pseudomonas putida KT2440	Environmental strain	Bagdasarian *et al.,* 1981
Pseudomonas aeruginosa PAO1	Wildtype burn wound isolate	Stieritz and Holder, 1975
Pseudomonas aeruginosa PA14	Wildtype burn wound isolate	Rahme *et al*., 1995
Pseudomonas aeruginosa PA14 Δ*anr*	Transposon mutant	Liberati *et al.,* 2006
Pseudomonas aeruginosa PA14 Δ*cupA1*	Transposon mutant	Liberati *et al.,* 2006
Pseudomonas aeruginosa PA14 Δ*pqsA*	Transposon mutant	Liberati *et al.,* 2006
Pseudomonas aeruginosa PBCLOp1	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp2	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp4	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp5	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp6	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp7	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp8	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp9	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp10	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp11	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp14	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp17	Burn wound isolate	This work
Pseudomonas aeruginosa PBCLOp18	Burn wound isolate	This work
Pseudomonas aeruginosa PBMHHp1	CF patient isolate	This work
Pseudomonas putida KT2440-pBBRMCS5-LITE	Bioluminescence	This work
Pseudomonas aeruginosa PAO1-pBBRMCS5-LITE	Bioluminescence	This work
Pseudomonas aeruginosa PBCLOp10-pHL320	Bioluminescence	This work

3.1.2. Media

3.1.2.1. Luria Bertani Medium (LB)

The LB medium (Sambrook *et al.*, 1989) was used as standard medium for all bacterial strains.

In 1000 ml H_2O:

10 g Bacto Tryptone

5 g Yeast extract

10 g NaCl

The pH was adjusted to 7.0 with 5M NaOH. Medium was autoclaved at 121^0C for 20 min. LB agar was obtained by adding 15 g per 1 l of liquid LB medium.

3.1.2.2. MacConkey Agar medium

MacConkey Agar was used as a medium for isolation and differentiation of *P. aeruginosa* strains from other possible bacteria present in clinical isolates. MacConkey agar selects Gram-negative bacteria and distinguishes between lactose fermenters and non-fermenters.

In 1000 ml H_2O:

8 g MacConkey agar (BD, Sparks, MD, USA)

Dissolved by heating, autoclaved at 121^0C for 20 min.

3.1.2.3. Columbia Blood Agar (BD)

Columbia Blood Agar (BD) plates were used for growth of *P. aeruginosa* to determine the haemolytic activity. Strains cultivated on these plates were further analysed by Vitek2 (Biomerieux, Marcy l'Etoile, France) system for resistance patterns with the standard Vitek procedures. The resistance report was made with the Vitek2 Systems Version 02.01n (Biomerieux).

3.1.2.4. ABT medium

The ABT medium with casamino acids was used for the experiment of *P. aeruginosa* response to protoanemonin, both for transcriptomic and proteomic analysis.

Materials and Methods

Stock solutions:

A10:

In 1000 ml H_2O:

20 g $(NH_4)_2SO_4$

60 g Na_2HPO_4

30 g KH_2PO_4

30 g NaCl

After dissolving, the pH should be adjusted to 6.4 and the solution autoclaved

BT:

In 900 ml H_2O:

1 ml of 1 M $MgCl_2$

1 ml of 0.1 M $CaCl_2$

1 ml of 0.01 M $FeCl_3$

1 ml of 2.5 mg/ml γ thiamin

After dissolving the solution should be autoclaved

Glucose:

20% glucose in H_2O

Casamino acids (CAS AA):

20% casamino acids

Glucose and CAS AA should be filter sterilized

Final ABT medium:

10 ml A10

90 ml BT

2.5 ml glucose (20 %)

2.5 ml CAS AA (20 %)

3.2. *In vitro* Bacterial growth

In vitro growth of *P. aeruginosa* clinical strains was performed in order to obtain non-virulent controls. The host site of the infection is rich in aminoacids, therefore in order to mimic the nutrient composition and physiological state of the cells during the infection the LB medium was chosen.

3.2.1. Planktonic growth in LB medium

All bacterial cultures were grown separately in 100 ml LB broth at 37^0C and 160 rpm in 1000 ml baffled Erlenmeyer flask until an optical density measured by 1:10 dilution of 0.6 (early stationary phase) at 600 nm. Afterwards, bacteria were harvested for RNA isolation as described below (see section 3.5.1).

3.2.2. Biofilm growth

Overnight pre-inoculum of *P. aeruginosa* was diluted to an optical density of 0.05 at 600 nm with 10% LB. The plastic Permanox® (Nunc, Rochester, NY, USA) slides were put into 100 ml of the diluted culture in 500 ml Erlenmeyer flask. Flasks were kept at 37^0C for 24h without shaking. Biofilm formation was observed on the plastic slides. Slides were washed two times in the fresh LB medium than dipped in RNAprotect reagent (Qiagen, Hilden, Germany) and biofilms were scraped by a cell scraper. The procedure followed was the same as for other RNA samples and is described in section 3.5.1.

3.2.3. ABT minimal medium growth with protoanemonin treatment

Overnight pre-inoculum of *P. aeruginosa* PAO1 growing in ABT minimal medium supplemented with 0.5% casamino acids was used to inoculate 200 ml of the same media to an optical density of 0.05 at 600nm. The *P. aeruginosa* PAO1 culture was incubated at 37^0C with shaking (160 rpm). When the culture reached middle exponential phase at the OD_{600} of 0.5 it was split into halves. One half was treated with 125 μM of protoanemonin and the second served as a control. Cultures were harvested for proteomic (see section 3.7) and transcriptomic analysis (RNAprotect reagent, Qiagen) (section 3.5.1) at the late

exponential/early stationary phase at the OD_{600} of 2.0. The experiment was performed in triplicate.

3.3. *In vivo* conditions

3.3.1. Burn wound sample collection

3.3.1.1. Criteria for choosing patients

The samples of *P. aeruginosa* infecting burn wounds were collected at the Centre for Burn Treatment (CLO) in Siemianowice Śląskie, Poland. The sampling protocol was approved by the Bioethical Commission of Silesian Medical Academy. Only patients with clinically confirmed *P. aeruginosa* burn wound infection were chosen for sample collection. To minimize invasion during sample collection, the samples were taken only from patients with liquid burn wound exudates. Liquid exudates were taken from burn wound surface using sterile forceps and immediately transferred to vials containing RNAprotect bacteria reagent (Qiagen) in order to prevent the degradation of RNA. The buffer with the sample was vortexed, incubated for 10 - 15 minutes at room temperature and centrifuged for 15 min at 4000 *x g*. The supernatant was discarded and the pellet frozen at -20^{0}C.

During sampling a standard swabs were also taken from the same area of the wound in order to collect the original clone of *P. aeruginosa*.

3.3.1.2. Sample transport

Frozen samples were transported on dry ice and stored at -70^{0}C until RNA extraction. Swab samples were transported at room temperature.

3.3.1.3. Clinical strain isolation

Swab samples were plated onto both LB and MacConkey agar plates immediately after arrival back to the laboratory. *P. aeruginosa* was differentiated from other bacteria by morphology difference (colony shape, colour, plate selection) taken into account previous

microbiological data obtained from the CLO. Table 3-2 summarizes all the bacterial species isolated from different patients.

Table 3-2: Strains isolated from patients with burn wound infection.

Patient	Microorganism isolated
P1	*Pseudomonas aeruginosa* PBCLOp1
	Proteus mirabilis
	Escherichia coli
P2	*Pseudomonas aeruginosa* PBCLOp2
	Acinetobacter baumannii
P3	*Proteus mirabilis*
P4	*Pseudomonas aeruginosa* PBCLOp4
P5	*Pseudomonas aeruginosa* PBCLOp5
	Staphylococcus aureus
P6	*Pseudomonas aeruginosa* PBCLOp6
	Acinetobacter baumannii
P7	*Pseudomonas aeruginosa* PBCLOp7
	Acinetobacter baumannii
	Proteus mirabilis
P8	*Pseudomonas aeruginosa* PBCLOp8
	Acinetobacter baumannii
P9	*Pseudomonas aeruginosa* PBCLOp9
P10	*Pseudomonas aeruginosa* PBCLOp10
P11	*Pseudomonas aeruginosa* PBCLOp11
P14	*Pseudomonas aeruginosa* PBCLOp14
	Acinetobacter baumannii
	Enterococcus feacalis
P15	*Pseudomonas aeruginosa* PBCLOp15
	Acinetobacter baumannii
P16	*Pseudomonas aeruginosa* PBCLOp16
	Proteus mirabilis
P17	*Pseudomonas aeruginosa* PBCLOp17

3.3.2. Cystic Fibrosis patient sputum sample collection

A sputum sample was kindly donated by 1 CF patient with their complete permission and knowledge. The patient had confirmed to have a chronic *P. aeruginosa* infection. The 3 ml of sputum was resuspended in RNAprotect bacteria reagent (Qiagen), incubated for 30 min. at room temperature and then centrifuged for 15 min at 4000 *x g*. The supernatant was discarded and the pellet frozen at -70^{0}C until further use.

Part of the sputum sample was not treated with RNAprotect in order to isolate the clinical strain of *P. aeruginosa*. The strain was isolated on Columbia agar following the selection on the MacConkey agar.

3.3.3. Tumour mouse model

3.3.3.1. Infection of tumour-bearing mice

Six-week-old female BALB/c mice were purchased from Harlan (Germany). All animal experiments were performed in accordance to the institute's and government regulations. Cells of the colon adenocarcinoma cell line CT26 (ATCC CRL-2638) were grown in IMDM medium (Gibco, Karslruhe, Germany) supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine and 10 mM HEPES. For injection into mice CT26 cells were trypsinised, washed and finally resuspended in phosphate-buffered saline (PBS). 10^6 cells were injected subcutaneously in the abdomen. Mice bearing tumours of approximately 4–6 mm diameter were intravenously injected with 5×10^6 CFU of bacteria suspended in PBS.

3.3.3.2. Recovery of bacteria from tissues

At selected time points, tissue samples were obtained (spleen, liver and tumour). Samples were transferred into 3 ml of sterile ice-cold PBS containing 0.1% (v/v) Triton X-100 and subsequently disrupted with a Polytron PT3000 homogenizer (Kinematica, Littau, Switzerland). For enumeration of bacteria, homogenates were serially diluted in PBS and streaked onto LB agar.

3.3.3.3. Bioluminescence *in vivo* imaging

Plasmids used for bioluminescence analysis:

- pHL320 (Loessner *et al*. unpublished), containing an optimised version of the green-emitting luciferase from click beetle present in pCBG99-Basic plasmid (Promega) and subcloned downstream of the constitutive promoter P_{bla}, transferred into the shuttle vector pUCP20 (West *et al.*, 1994b).

- pBBRMCS5-LITE (Marques *et al.*, 2005) containing the *luxCDABE* gene cassette of *Photorhabdus luminescens* from plasmid pLite27 (Marincs & White, 1994) under the constitutive P_{lac} promoter, introduced into broad-host-range vector pBBR1MCS-5 (Kovach *et al.*, 1995).

In vivo visualisation of tumour infection was performed using transformed strains *P. aeruginosa* PBCLOp10-pHL320, *P. aeruginosa* PAO1-pBBRMCS5-LITE and *P. putida* KT2440-pBBRMCS5-LITE. Mice were infected using the method mentioned above. Mice infected with the strain PBCLOp10-pHL320 received an intraperitoneal dose of 3 mg D-luciferin substrate (Caliper Life Sciences, Hopkinton, MA, USA) diluted in 100 µl PBS before analysis. Subsequently, mice were anaesthetised with 2 % isoflurane using the XGI-8 gas anesthesia system (Caliper Life Sciences). Pseudocolored images of photon counts and photographic images were obtained according to the instructions of the manufacturer. The software Living Image 2.5 (Caliper Life Sciences) was used for image analysis and quantification of emission intensities which are expressed as radiance (p s-1 cm-2 sr-1).

3.3.3.4. Tumour collection for *P. aeruginosa* RNA extraction

Three days post-infection (p. i.) mice were necrotised and infected tumours prepared for stabilization of bacterial RNA in the following way: the tumours were cut into 2-4 pieces, put on the nylon filter for cell cultures (70 µm pore size) and suspended in with 2 ml of RNAproctect Bacteria Reagent (Qiagen) on 2 cm petri dish. The tumour was squeezed through the membrane with sterile spatula. The resulting mixture in RNAprotect reagent was collected to the 2 ml reaction tube and centrifuged for 5 min at maximum speed. The pellet was frozen at -70°C for further RNA extraction performed as described in section 3.5.1.

3.3.4. Lettuce infection model

3.3.4.1. Infection of lettuce leaves

The protocol for using romaine lettuce leaves as a model of *P. aeruginosa* infection and *P. putida* KT2440 injection as control, was performed as described previously (Filiatrault *et*

al., 2006). *P. aeruginosa* strains were grown aerobically overnight at 37°C and *P. putida* KT2440 at 30°C in LB broth, washed twice with 10 mM $MgSO_4$, and diluted in sterile $MgSO_4$ to a bacterial density of 1 x 10^8 CFU/ml. Lettuce leaves (Mini-Roma lettuce purchased commercially) were prepared by washing with distilled H_2O and 0.1% bleach. Lettuce mid-ribs were inoculated with 10 µl of bacterial suspension at a density of 1 x 10^8 CFU/ml (corresponding to ~1 x 10^6 bacteria) by injecting the end of the plastic tip into the rib and placed in plastic containers containing Whatman paper moistened with 10 mM $MgSO_4$. Lettuce was incubated at 37°C or 30°C, and symptoms were monitored daily over the course of 5 days. As a negative control, lettuce leaves were inoculated only with 10 mM $MgSO_4$. A separate lettuce leaf was used for each strain. The experiments were repeated three times.

3.3.4.2. Sample collection for RNA extraction

Five days post infection the 2 cm^2 piece from original place of injection site was cut out from the leaf and putted to 15 ml reaction tube with 3 ml RNAprotect Bacteria Reagent (Qiagen). The sample was vortexed for 30 seconds and the solid parts of the plant tissue discarded. Resulting mixture was incubated for 5 min at room temperature and centrifuged for 15 min at 4000 *x g*. The supernatant was discarded and the pellet frozen at -70°C for further RNA extraction performed as described in section 3.5.1.

3.4. DNA methods

3.4.1. Genotyping of clinical *P. aeruginosa* strains

The genotyping of *P. aeruginosa* isolates was performed according to protocols and techniques developed at the Klinische Forschergruppe led by Prof Burkhard Tümmler at Medizinische Hochschule Hannover (MHH). The method is based on binary microarray (Wiehlmann *et al.*, 2007), which consists of 58 targets representing the core and the accessory genome. The core genome is analysed by 13 single-nucleotide polymorphisms (SNPs) at seven conserved loci and two multiallelic loci (flagelin *fli*C and pyoverdine receptor *fpv*A). The accessory genome is tested with set of genetic markers that identify 10 genomic islets and six types of genomic islands (Wiehlmann *et al.*, 2007). All targets are amplified

from the *P. aeruginosa* colonies by the multiplex primer extension reaction with random labelling performed by incorporation of biotin-16-dUTP. The multiplex amplificate is hybridised under the high stringency with the oligonucleotide microarray of the target sequences that is inserted into the tip of a standard Eppendorf-like microtube. The hybridisation signals are automatically converted to the multilocus genotype. The 16 binary SNP genotypes are represented by a four-digit hexadecimal code. The 16 SNPs are divided into four groups of four SNPs each, and the 16 possible combinations in each group are differentiated by 16 characters: 0–9, A–F.

The illustration of clonal diversification was generated using an eBurst algorithm (Feil *et al.*, 2004). The clinical strains were analysed together with the database of MHH which contains more than 1700 isolates from 658 independent sources (both clinical and environmental).

3.4.2. Plasmid DNA isolation

Plasmid DNA was extracted using QIAprep Spin Miniprep Kit (Qiagen) following supplier instructions. The size and purity of plasmid DNA was tested on 1% agarose gels prefaced with restriction enzyme digestion. The size was checked in comparison to 1kb DNA ladder (Invitrogen)

3.4.3. Agarose gel electrophoresis

Agarose gel electrophoresis was performed according to Sambrook *et al.*, (1989). Each DNA sample was mixed with the 1/5 volume of loading buffer and loaded on a 1% (w/v) gel. The TAE buffer was used as a running buffer.

3.4.4. *P. aeruginosa* electrocompetent cells

P. aeruginosa was grown on the LB agar plate at 37°C. Freshly grown colonies were transferred from plate to 1.5 ml reaction tube and resuspended in 1 ml sterile H_2O. Bacteria were washed 3 times with 500 µl of H_2O. Finally, cell pellet was dissolved in 20 µl of H_2O.

3.4.5. Electroporation

Plasmid DNA was mixed with electrocompetent cells of *P. aeruginosa* or *E. coli* TOP10, put to fresh electroporation cuvette. Electroporation was done in following settings: potential 2.5 kV (*Pseudomonas*) or 1.8 kV (*E. coli*), capacity of 25 μFD and resistance of 200Ω.

Directly after the electroporation, 800 μl of LB medium was added to the mixture and transferred to sterile reaction tube. The mixture was incubated at 37°C for 1 h with 180rpm shaking. After incubation 50 or 100 μl of bacterial suspension was plated on LB agar plates (50 μg/ml Gentamycin or Carbenicillin 50 μg/ml) and incubated overnight at 37°C.

3.4.6. *P. putida* KT2440 conjugation

Plasmid pBBRMCS5-LITE was transformed to *P. putida* KT2440 by triparental mating using the *E. coli* TOP10 harbouring plasmid pBBRMCS5-LITE as a donor strain and *E. coli* HB101(RK600) as a helper strain. 10 ml of LB medium, containing adequate antibiotic (Gentamycin 10 μg/ml for the donor, chloramphenicol 20 μg/ml for the helper and ampicilin 100 μg/ml for the host), was inoculated with each of strains and incubated at 37°C or 30°C until the cultures reached the optical density of OD_{600nm} = 0.8-1.0. In 2 ml sterile reaction tube, the 2 ml of host strain *P. putida* KT2440 were centrifuged for 1 min at 7000 rpm and the supernatant was discarded. To the pellet, 1 ml of helper strain *E. coli* HB101(RK600) and 500 μl of donor strain solution were added (equilibrated so there is ratio of 2 : 1 : 0.5 of host, helper and donor) and centrifuged again for 1 min at 7000 rpm. The mixture was washed twice with LB, to remove the traces of antibiotics, and resuspended in 100 μl of LB medium without antibiotics. The conjugation suspension was disposed in the sterile nitrocellulose filter (0.45 μm diameter) and placed on LB agar plate. After overnight incubation at 30°C the bacterial cells from the filter were resuspended in 2 ml of LB medium and serial dilutions were plated on the LB agar with gentamycin and ampicillin in order to select the *P. putida* KT2440 transconjugants harbouring the pBBRMCS5-LITE vector.

3.5. Transcriptomic analysis

3.5.1. Total RNA isolation

Analysis of gene expression of the pathogen directly in infection site is limited by various factors connected with RNA extraction. The RNA extracted from clinical samples will most likely be a mixture of the bacterial RNA with the host RNA. Due to the low quantity of RNA, the procedure for isolation and preparation was optimized for the quality of microarrays on the samples collected from burn wound patients.

3.5.1.1. RNA isolation

All samples for the RNA measurement were stabilised with RNAprotect bacteria reagent (Qiagen). Samples were mixed with this reagent, incubated for 5 - 15 min at room temperature and centrifuged for 15 min at 4000 *x g* in the 4°C. Supernatant was discarded and pellet frozen in -70°C. In deep freezer the RNA should remain intact for up to one month. Prior to the RNA extraction samples were thawed on ice.

RNA isolation was performed using RNAeasy Mini Kit (Qiagen) according to manufacturer's instructions with some minor modifications: samples were treated with 600 µl of TE buffer containing 1 mg/ml Lysozyme, incubated for 10 min with periodic vortexing every 2 min for 15 sec. Then, 1050 µl of RLT buffer containing 1% β-mercaptoethanol was added. The sample was vortexed and centrifuged for 2 min at maximum speed (ca. 13000 rpm). The supernatant was transferred to a fresh 15 ml tube and 750 µl of absolute ethanol was added. The sample was loaded onto a spin column where the DNA was digested using RNase-free DNase I. The RNA was then eluted twice from each column with 50 µl and then 30 µl of RNase-free water. Eluted RNA was treated a second time with DNase I to ensure that all traces of genomic DNA were removed. The isolated RNA was stored at -70°C.

3.5.1.2. RNA quality measurement

The yield of the isolated RNA was measured by light absorption at 260nm in Eppendorf photometer. The quality and possible degradation was checked by formaldehyde gel electrophoresis or by 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). Using photometrical measurements it is possible to estimate the quality of RNA by calculating the ratio of absorption between 260nm and 280nm. The RNA is considered pure and the least degraded when the A_{260}:A_{280} ratio is within 1.7 and 2.1. (Sambrook *et al.,* 1989)

3.5.1.3. Formaldehyde gel electrophoresis

In order to obtain a better resolution of the RNA on an agarose gel, electrophoresis was performed on a formaldehyde-agarose (FA) gel. The composition of loading buffer and agarose gel (see recipes below) prevent RNA from forming secondary structures.

<u>1.2% FA gel consists of:</u>

1.2 g agarose, 10 ml 10x Running buffer, 100 ml RNase-free water, 1.8 ml of 37% formaldehyde and 1 µl of 10 mg/ml ethidium bromide solution.

<u>10x FA gel buffer:</u>

200 mM 3-[N-morholino]propanesulfonic acid (MOPS), 50 mM sodium acetate, 10 mM EDTA, pH adjusted to 7 with 5M NaOH.

<u>1x FA gel running buffer:</u>

100 ml 10x FA gel buffer, 20 ml 37% formaldehyde, 880 ml RNase free water.

<u>5x RNA loading buffer:</u>

16 µl saturated aqueous bromophenol blue solution, 80 µl 500 mM EDTA (pH 8.0), 720 µl 37% formaldehyde, 2 ml 100% glycerol, 3084 µl formamide, 4 ml 10x FA gel buffer, RNase-free water up to 10 ml.

The casted gel is first equilibrated by running without RNA at 7 V/cm for 30 min. The samples are mixed with 5x loading buffer and incubated for 5 min at 65°C, then cooled on ice and

transferred to the gel wells. The samples run at 7 V/cm with the 0.5-10 kb RNA ladder (Invitrogen) as the standard.

3.5.2. Bacterial RNA enrichment

Since the extracted RNA from the *in vivo* samples contained both bacterial and eukaryotic RNA (i.e. human or mouse), the samples had to be enriched for bacterial RNA. This was achieved with the MicrobEnrich Kit® (Ambion, Austin, TX, USA). The basis of the kit is the hybridisation of the eukaryotic ribosomal RNA and the messenger RNA to the magnetic beads. Hybridisation occurs between specific 18S and 28S RNA regions and polyA tails of eukaryotic mRNA. Since there are reports that bacterial mRNA posses poly adenylated (polyA) tails to some extent (Saravanamuthu *et al.*, 2004) all of the samples including the controls were treated for enrichment.

That prevents obtaining false positives in microarray analysis. Figure 3-2 presents Agilent BioAnalyzer results of total RNA before enrichment and after.

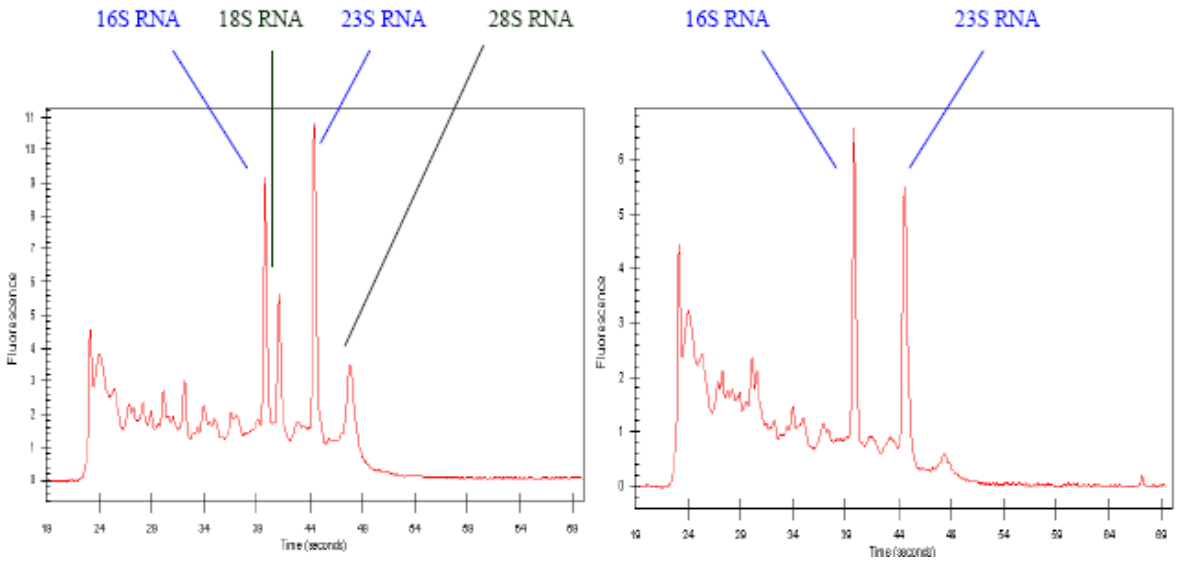


Figure 3-2: Agilent bioanalyzer results of bacterial RNA enrichment from sample p10; a) samples before enrichment, peaks from bacterial ribosomal RNA (16S and 23S) are seen together with eukaryotic ribosomal RNA (18S and 28S); b) sample p10 after enrichment, only bacterial ribosomal signals are detected

3.5.3. RNA amplification

One of the main bottlenecks in the transcriptomic analysis of host-pathogen interactions is the amount of bacterial RNA that can be isolated from the sample taken from the site of infection. The well established *P. aeruginosa* GeneChip® from Affymetrix (Santa Clara, CA, USA) requires 10 µg of RNA per microarray. Even with burn wound infections, where the amount of bacterial cells is relatively large this amount was never obtained. A promising way to overcome this obstacle was with the use of bacterial RNA amplification. This was performed using the MessageAmp Bacteria Kit (Ambion). The procedure consists of the following steps: i) total enriched bacterial RNA is treated with an enzyme polyadenylation polymerase to produce polyA tails, ii) single stranded cDNA is produced using reverse transcriptase and oligo dT primers, iii) second strain cDNA is produced, and iv) double stranded cDNA serves as a template for *in vitro* transcription using T7 RNA polymerase and T7 oligonucleotides. During the *in vitro* transcription reaction modified nucleotides where used: biotin-11-CTP (PerkinElmer Life Sciences, Waltham, MA, USA) and biotin-16-UTP (Roche Applied Science, Basel, Switzerland). As a result we obtained antisense biotinilated RNA, ready to use for GeneChip® hybridisation.

3.5.4. Affymetrix microarrays analysis

The use of amplified RNA is common with eukaryotic microarrays, but the original procedure for *P. aeruginosa* GeneChip® (Affymetrix) was prepared only for single stranded terminally labeled cDNA procedure. Comparing to the standard procedure (without amplification), amplifying RNA for microarray analysis does not change the final outcome as reported by Francois *et al.,* (2007). The hybridisation and washing steps were performed in the Affymetrix Array facility in HZI Braunschweig (Dr. Robert Geffers). Because the RNA was amplified, some changes were introduced to the original Affymetrix protocol, such as that the RNA was fragmented using 5x fragmentation buffer instead of DNaseI treatment. The total amount of amplified and fragmented RNA used per chip was 6.5 µg.

The process of sample preparation for each chip was as follows:

- The RNA from each single condition was pooled together after the enrichment.
- Amplification step was performed on each condition
- Amplified RNA was hybridised onto duplicate microarray chips in order to have technical replicates

Initial steps of data analysis were done in Array facility using Affymetrix Microarray Suite Software 5.0 with default parameters. Once raw data files of scanned pictures were obtained further bioinformatic analysis was made as described in section 3.7.

3.6. Statistical analysis

The statistical approach used to analyse the microarray data in this work is indicated in red in Figure 3-3.

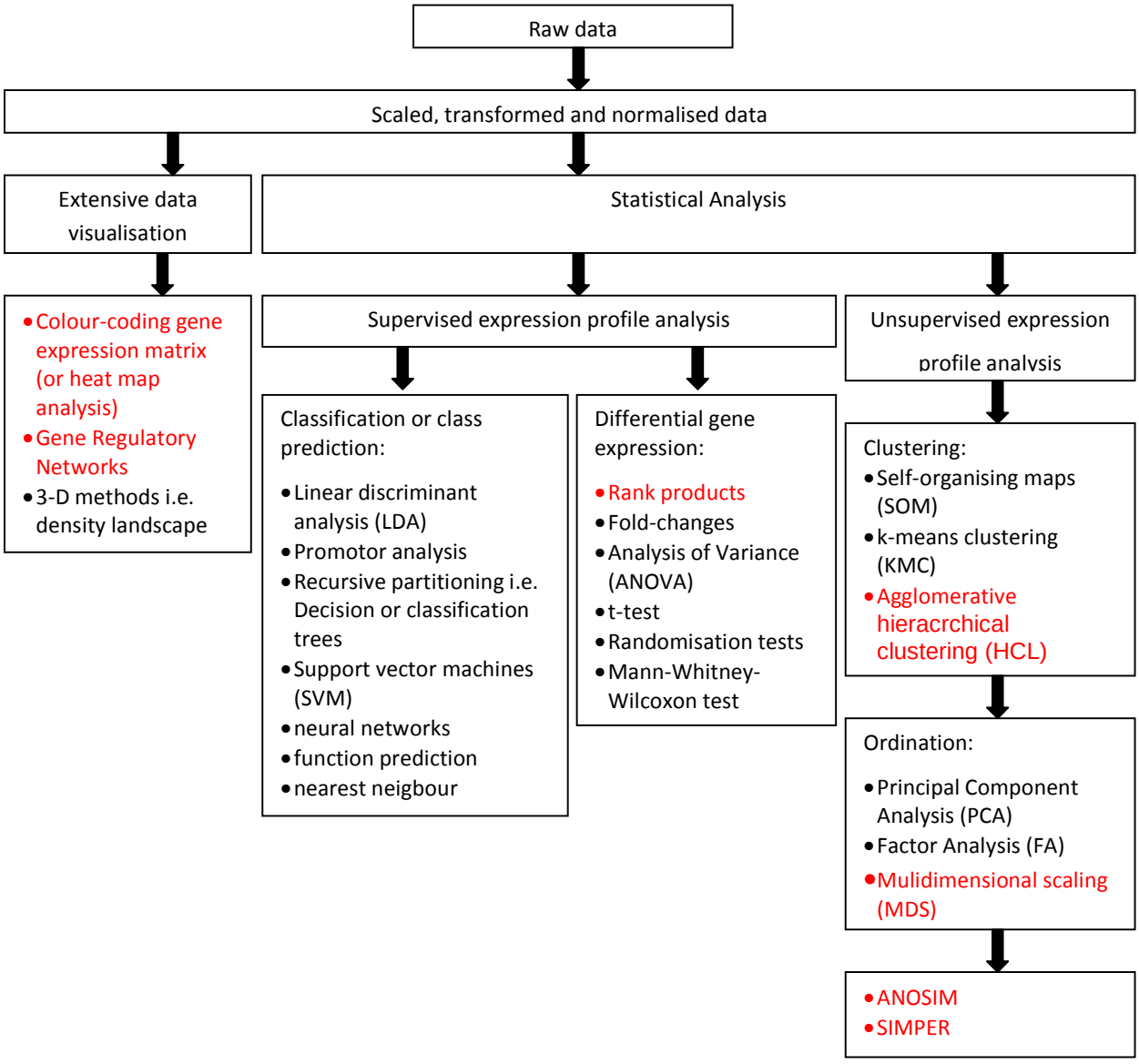


Figure 3-3: Scheme of the typical microarray data analysis approach using supervised and unsupervised routines. Those routines used for this work are indicated in red.

3.6.1. Microarray data normalisation

All of the analyses mentioned in sections 3.6.2 and 3.6.3 were performed with applications belonging to appropriate software packages from Bioconductor microarray analysis suite (Gentleman *et al.*, 2004). The quality of all chips was assessed by fitting a linear model to the probe level data using the function "fitPLM" from the "affyPLM" package. Subsequently, the distribution (boxplots) of RLE (Relative Log Expression) and NUSE (Normalised Unscaled Standard Errors) were manually analysed. Expression values were computed using the "Robust Multichip Average" algorithm (Irizarry *et al.*, 2003). The computations were done using function "rma" from the package "affy".

3.6.2. Differential Expression

As the number of replicates was low, the "Rank Products" algorithm was used to identify differentially expressed genes (Breitling *et al.*, 2004). It has been shown that this algorithm performs well when the number of replicates is low (Jeffery *et al.*, 2006). The algorithm addresses the multiple testing problems by calculating for every gene an estimate of percentage of false-positives (pfp), if this gene and all genes with lower pfp would be considered as significantly differentially expressed. Thus, it is an estimate of False Discovery Rate (FDR). The value of 0.05 was accepted as a cut-off for pfp. The computations were performed using function Rpadvance from the "RankProd" package. A list of significantly differentially expressed genes was created for every pair of growth conditions (burn wound, lettuce, mouse tumour, planktonic, biofilm, CF patient infection).

3.6.3. Hierarchical clustering

On all genes that showed significant expression change in at least one pairwise comparison between the experiments (1677 genes) hierarchical clustering was performed (correlation was used as the distance measure, the clustering was performed with the "hclust" function from the R software suite, http://www.r-project.org/). Subsequently, the outcome of the clustering was used to identify groups of genes following similar pattern of expression

changes (222 groups were created). The expression values were averaged within each of the groups to form for each of them a group-wide expression pattern. Finally, the hierarchical clustering was performed on the groups, following the same procedure as previously. The dendrogram, together with the heatmap composed of the group-wide expression values were plotted.

3.6.4. Multivariate statistics

For the unsupervised approach of gene expression visualisation the non-metric multidimensional scaling (nMDS) routine was used. The nMDS was shown to be useful to represent high dimensional data in a low dimensional space with preservation of the similarities between data points. When the data configuration is Euclidean, MDS is similar to principle component analysis (PCA), which can remove inherent noise with its compact representation of data (Tzeng *et al.*, 2008). However, PCA is a linear algebraic routine which is restricted to the domain of Euclidean input spaces where variance is a properly defined concept (Strickert *et al.*, 2007). It was shown that nMDS is more powerful to present data in low-dimensional spaces (Taguchi & Oono, 2005, Strickert *et al.,* 2007).

Non-parametric multivariate statistical analysis was performed using PRIMER v6 (Plymouth Marine Laboratory, UK) (Clarke, 1993). The multivariate routines applied here were non-metric multidimensional scaling (nMDS), analysis of similarity (ANOSIM) and similarity percentage analysis (SIMPER) described previously (Wilber *et al.*, 2007). Sample-similarity matrices were calculated from gene expression level data using the Euclidean distance (the commonly used dissimilarity measure) (Butte, 2002). In this case, each gene is a point in multidimensional space where each axis (dimension) is a separate infection condition and the coordinate on each axis is the level of gene expression (Wen *et al.*, 1998). MDS was used to ordinate each sample with each other sample based on the comparative expression level of 1677 genes, which were those that were differentially regulated in at least one comparison between *in vivo* and *in vitro* conditions. The program used 50 random restarts to find the optimal ordination and stress value. The stress value of each MDS value plot represents an estimation of the quality of fit of the data. A stress value below 0.1

corresponds to an ideal ordination indicating that there is no real prospect of misinterpretation, a stress value below 0.2 corresponds to a useful indicating a valuable 2D representation and stress value higher than 0.2 indicates that the plot is close to random (Clarke & Warwick, 2001).

Analysis of similarity (ANOSIM) was used to test for significant differences between predefined groups of samples (burn wound infection, mouse tumor infection, lettuce infection, biofilm and planktonic growth). It produces a test statistic (R) which can range from -1 to 1 and is a useful comparative measure of the degree of separation between groups. An R-value higher than 0 indicates the null hypothesis is true, and that there are no significant differences between groups. R usually falls between 0 and 1 which indicates some degree of distinction between groups. An R-value lower than 0.25 indicates barely separable groups, a R-value higher than 0.5 indicates clear differences between groups with some degree of overlap, and a R-value higher than 0.75 indicates well separable groups (Wilber *et al.,* 2007). When testing more than two groups a global R test indicates if differences between groups are present that may be worth examining further. If the Global R is not significant, generally no further interpretation is permissible. If it is significant, R-values for each pairwise comparison should be examined. Similarity percentages analysis (SIMPER) was used to determine which of the genes mostly contribute to the similarity and dissimilarity between different infection conditions.

3.7. Proteomic analysis

3.7.1. Extracellular proteins extraction

Supernatants obtained after centrifugation of the bacterial cultures at 6000 *x g* for 15 min at 4°C were passed through a 0.2-µm-pore-size filter. Deoxycholic acid (sodium salt) was added to a final concentration of 0.2 mg/ml. After 30 min of incubation on ice, the proteins were precipitated by addition of trichloroacetic acid to a final concentration of 10% (w/v) and incubated at 4°C overnight. After centrifugation at 10000 *x g* for 30 min the precipitated proteins were resuspended in an appropriate amount of rehydration solution (7 M Urea,

Serdolit, 2 M Thio-urea, 4% w/v CHAPS, 20 mM Trizma base). Protein extracts were re-precipitated with 2D Clean-Up Kit (Amersham Bioscience, Pittsburgh, PA, USA), resuspended in 200 µl of rehydratation solution and stored at -70°C until further use.

3.7.2. Two-dimensional gel electrophoresis

3.7.2.1. Isoelectric focusing

Analytical determinations were carried out with 100 µg of protein mixture determined by Bradford test (Bio-Rad protein assay, Bio-Rad, Hercules, CA, USA), diluted up to 300 µl with rehydration solution in the presence of ampholytes and under reducing conditions on ReadyStrip IPG strips, 17 cm, pH 4-7 (Bio-Rad). Passive rehydration was carried out for 2h at 20°C on the focusing tray. Samples were covered with silicon oil to avoid dehydration. Active rehydration was performed at 50 V for 12 h. Isoelectric focusing was done at a final voltage of 10000 V on Protean®IEF cell (Bio-Rad) until reaching 75 kWh. Focused samples were stored at -70°C until the second dimension step.

3.7.2.2. Second dimension: equilibration and SDS-PAGE

Focused ReadyStrip IPG strips were equilibrated first in equilibration buffer containing Urea 6 M, Trizma Base 0.375 M (pH 8.6), Glycerin 30% v/v, SDS 2% w/v and DTT 2% w/v and later in the same buffer replacing DTT with iodoacetamide 2.5% w/v. After equilibration, second-dimension separation was performed on 12-15% gradient SDS polyacrylamide 20x20 cm gels with the focused sample embedded in 0.5% IEF agarose in a Protean Plus Dodeca Cell (Bio-Rad) at 100 V overnight. The gels were fixed in 10% trichloroacetic acid solution for a minimum of 3 h, stained with 0.1% w/v Coomassie™ Brilliant Blue G-250 solution overnight and finally destained with distilled water. Images of the 2-DE gels were captured with a molecular imager GS-800 calibrated densitometer (Bio-Rad) and processed using Z3 image analysis software (Compugen, San Jose, CA, USA) for protein differential expression analysis.

3.7.3. Protein differential expression

Differential expression (DE) analysis was done using Z3 image analysis software version 3.0.7 (Compugen). Briefly, scanned gel images were saved in grayscale, 300 dpi with no adjustments. Images were first subject to automatic spot detection, with automatic minimum spot contrast and manually adjusted minimum spot area (100, arbitrary units). Detected spots were edited manually in order to obtain an optimal pattern. A three independent replicates for each reference condition were analysed and combined using the Raw Master Gel (RMG) algorithm. Comparison of the RMG reference gel was performed in triplicate, that were independently wrapped and matched to the reference RMG to obtained at least three independent DE sets. DE was defined as the ratio of spot expression in a comparative image to the expression of a corresponding spot in a reference image. Upregulation corresponds to a two-fold or higher DE values and downregulation to 0.5-fold or lower DE values.

3.7.4. Protein identification

Protein spots were excised manually from the gels. Spots were destained, and digested overnight using sequence grade modified trypsin (Promega, Madison, WI, USA). The peptides were eluted and desalted with ZipTip® (Millipore, Bedford, MA, USA). For MALDI-ToF analysis, the samples were loaded along with α-cyano-4-hydroxycinnamic acid matrix. The target was then analysed using an Ultraflex II ToF (Bruker Daltonics Inc. Billerica, MA, USA) and resulting spectra were used for Peptide Mass Fingerprint (PMF), analysed using FlexAnalysis 2.0 and Biotools 2.2 software (Bruker Daltonics Inc.). Database search was carried out on NCBInr database using Profound version 4.10.5 (Proteometrics, New York, NY, USA).

4. Results

4.1. Analysis of *P. aeruginosa* burn wound isolates

4.1.1. Resistance patterns

All fourteen *P. aeruginosa* strains isolated from burn patients were characterised for their susceptibility to 20 common antibiotics (Table 4-1) using the VITEK2 system (Biomerieux). The difference in resistance to these antibiotics was compared to *P. aeruginosa* strains PAO1 and PA14. Among the commonly used anti-pseudomonal agents are: the beta-lactams (piperacilin, cefoperazone, ceftazidime, cefepime, imipenem and meropenem); fluoroquinolons (ciprofloxacin and levofloxacin); and the aminoglicosides (gentamycin, tobramycin and amikacin) (Rossolini & Mantengoli, 2005). However, clinical strains acquire resistance to such agents, becoming multitresistant and complicating the eradication from nosocomialy infected patients.

The isolated *P. aeruginosa* strains from the Centre for Burn Treatment in Sieminowice Śląskie (CLO) presented a broad spectrum of antibiotic resistance, with some exhibiting a similar pattern to PAO1 and PA14 and others to the more multiresistant strains. For example, isolates PBCLOp5, PBCLOp11 and PBCLOp14 exhibited a resistance pattern similar to PAO1 and PA14 whereas isolates PBCLOp4, PBCLOp6, PBCLOp7 and PBCLOp15 showed resistance to piperacilin as well as to aminoglicosides and fluoroquinilons. The strains that have resistance to more antibiotics were isolates PBCLOp10 and PBCLOp17, which were resistant to both piperacilin and the combination of piperacilin with beta-lactamase inhibitor tazobactam. PBCLOp10 is susceptible to fluoroquinolones, while PBCLOp17 is only susceptible to carbapenem antibiotic meropenem, making it the most multiresistant of these 14 clinical strains.

Table 4-1: Antibiotic resistance patterns of clinical *P. aeruginosa* strains. R = Resistant, S = Susceptible and I = Intermediate. R in bold represents the result different than in the wildtype strains.

	Beta lactams													Aminoglicosydes		Quinolones			Glycylines	Sulfonamides
	Penicillins				Cephalosporins								Carbapenem							
Strain:	Ampicilin	Ampicillin/Sublactam	Piperacillin	Piperacillin/Tazobactam	Cefazolin	Cefuroxime	Cefuroxime Axetil	Cefoxitin	Cefpodoxime	Cefotaxime	Ceftazidime	Cefepime	Meropenem	Gentamycin	Tobramycin	Nalidixic Acid	Ciprofloxacin	Levofloxacin	Tigecycline	Trimethoprim*
PAO1	R	R	S	S	R	R	R	R	R	I	I	S	S	S	S	R	I	I	R	R
PA14	R	R	S	S	R	R	R	R	R	I	I	S	S	S	S	R	S	S	R	I
PBCLOp1	R	R	S	S	R	R	R	R	R	R	S	S	S	**R**	**R**	R	**R**	**R**	R	R
PBCLOp2	R	R	S	S	R	R	R	R	R	R	S	I	S	**R**	**R**	R	**R**	**R**	R	R
PBCLOp4	R	R	**R**	S	R	R	R	R	R	R	S	I	S	**R**	**R**	R	**R**	**R**	R	R
PBCLOp5	R	R	S	S	R	R	R	R	R	R	S	S	S	S	S	R	S	S	R	R
PBCLOp6	R	R	**R**	S	R	R	R	R	R	R	S	I	S	**R**	**R**	R	**R**	**R**	R	R
PBCLOp7	R	R	**R**	S	R	R	R	R	R	R	S	I	S	**R**	**R**	R	**R**	**R**	R	R
PBCLOp8	R	R	S	S	R	R	R	R	R	R	S	S	S	**R**	S	R	S	S	R	R
PBCLOp9	R	R	S	S	R	R	R	R	R	R	**R**	S	S	**R**	R	R	I	I	R	R
PBCLOp10	R	R	**R**	**R**	R	R	R	R	R	R	I	I	**R**	**R**	**R**	R	S	S	R	R
PBCLOp11	R	R	S	S	R	R	R	R	R	R	I	I	S	I	S	R	**R**	I	R	R
PBCLOp14	R	R	S	S	R	R	R	R	R	R	S	S	S	S	S	R	S	S	R	R
PBCLOp15	R	R	**R**	S	R	R	R	R	R	R	S	I	S	**R**	**R**	R	**R**	**R**	R	R
PBCLOp16	R	R	**R**	S	R	R	R	R	R	R	S	I	S	**R**	S	R	S	S	R	R
PBCLOp17	R	R	**R**	**R**	R	R	R	R	R	R	**R**	**R**	S	**R**	**R**	R	**R**	**R**	R	R

*Full name: Trimethoprin/Sulfamethoxazole

4.1.2. Genotyping of *P. aeruginosa* strains

Genotyping analysis was used to determine the population structure of *P. aeruginosa* at the CLO. Figure 4-1 presents the genetic tree aligning approximately 1700 *P. aeruginosa* isolates from Medizinische Hohschule Hannover with the 14 burn wound strains collected in this work. Well studied strains of *P. aeruginosa* are indicated as blue and the isolated strains as green. This phylogenetic tree shows that the six isolates PBCLOp1, 2, 4, 6, 7, 15 are representatives of the same clone. Interestingly, since sampling of strains PBCLOp1 and PBCLOp15 was performed two years apart it can be assumed that this clone is permanently residing at the CLO. Also, the clones PBCLOp4, 6, 7 and 15 exhibited resistance to the same antibiotics (Table 4-1), while strains PBCLOp1 and PBCLOp2 differed only in piperacilin resistance. Strains PBCLOp5, 10, 14 and 17 are the other group of highly similar but not identical clones. They are closely related to the *P. aeruginosa* strain CHA which is a highly virulent isolate from a CF patient in France (Morales *et al.*, 2004). Strains PBCLOp8 and 11 are also closely related to each other. PBCLOp9 and 16 are different from other strains in the database.

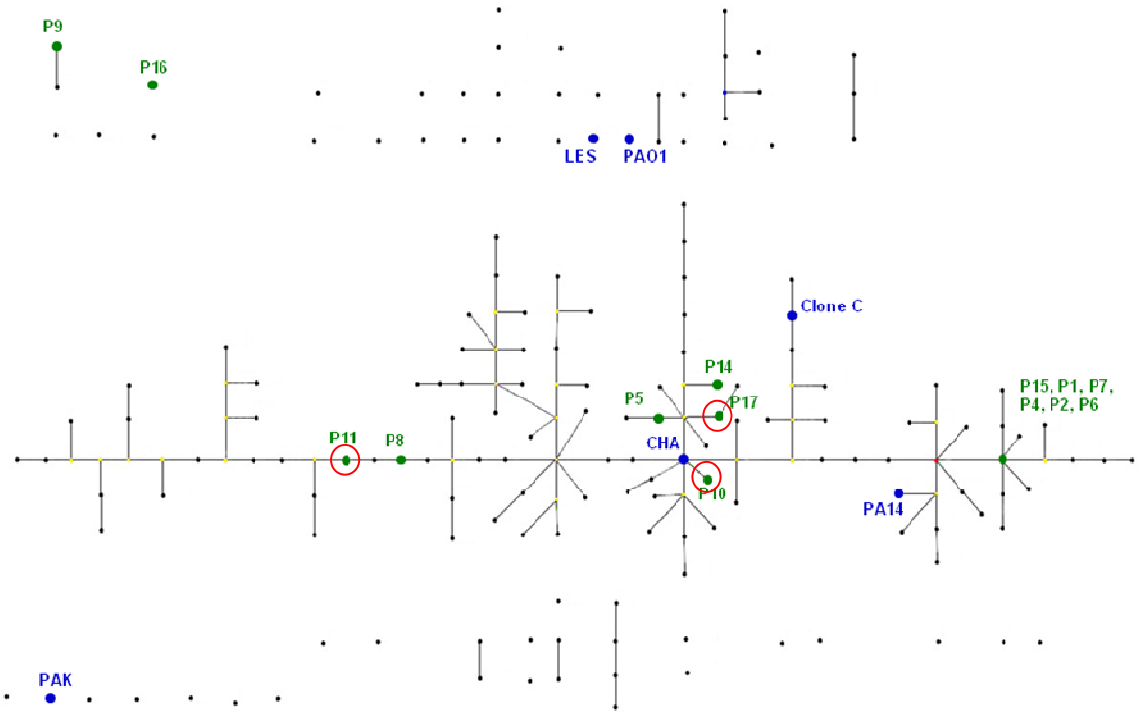


Figure 4-1: Genetic tree constructed with the eBurst algorithm. The branches joined together have a difference of one SNP. The clones which have a difference greater than one SNP are not connected. Blue dots indicate strains that are previously reported, while green dots indicate strains that were isolated in this work. Red circles indicate clones used for transcriptional profiling in this work.

4.2. Elucidating the genetic programs of *P. aeruginosa* in different infection conditions

The principal aim of this work was to assess the difference of the genetic programs between the non-virulent and virulent conditions by comparing their transcription profiles. While the burn wound infection is the key *in vivo* condition addressed in this work, it is followed by the preliminary analysis of a clinical strain from a CF patient, and 2 models of infection: the plant infection model performed on lettuce leaves and the novel tumour mouse infection model. The non-virulent conditions serving as controls were planktonic and biofilm growth on rich LB broth. The differential gene expression is calculated by comparing each *in vivo* sample to both of these non-virulent conditions.

4.2.1. Burn wound infection

A number of preliminary tests assessing microarray hybridisation revealed that successful analysis can only be obtained when using pure cultures of *P. aeruginosa*. That is, even when bacterial RNA was extracted in sufficient amounts from clinical samples, only those samples which comprise homogenous *P. aeruginosa* could be used. Thus, these isolates were PBCLOp10, PBCLOp11 and PBCLOp17 and are all different clone variants of *P. aeruginosa* (Figure 4-1). Therefore, the data obtained was not clone specific but incorporates distinctive *P. aeruginosa* characteristics.

4.2.1.1. Transcription profiling

Table 8-1 on page 146 in appendix shows the differentially expressed genes that have a percent-of-false positive (pfp) value lower than 0.05. When comparing the burn wound infection with the 2 control conditions of planktonic and biofilm growth, there were 244 and 232 genes upregulated, and 334 and 270 genes downregulated, respectively. This whole list of significantly regulated genes provides a comprehensive overview on the state of *P. aeruginosa* cells during a burn wound infection. It is not possible to describe all of these genes here, so only those genes with higher changes and also those that appear interesting from the point of view of infection will be further presented and discussed (Table 4-2).

Table 4-2: Summary of those genes that were differentially expressed (pfp < 0.05) and that also have a higher change or appear interesting in the context of burn wound infection.

		Fold change compared to:		
PA Number	Gene	Planktonic	Biofilm	Product Name
PA0102		7.46	12.11	probable carbonic anhydrase
PA0105	*coxB*	-12.23		cytochrome c oxidase, subunit II
PA0106	*coxA*	-35.41		cytochrome c oxidase, subunit I
PA0107		-34.07		conserved hypothetical protein
PA0108	*coIII*	-69.43	-3.09	cytochrome c oxidase, subunit III
PA0472	*fiuI*		3.93	probable sigma-70 factor, ECF subfamily
PA0652	*vfr*	2.36		transcriptional regulator Vfr
PA0707	*toxR*	4.34	4.16	transcriptional regulator ToxR
PA0762	*algU*		2.75	sigma factor AlgU
PA0763	*mucA*		4.78	anti-sigma factor MucA
PA1300			5.49	probable sigma-70 factor, ECF subfamily
PA1581	*sdhC*	3.13		succinate dehydrogenase (C subunit)
PA1582	*sdhD*	3.10		succinate dehydrogenase (D subunit)
PA1787	*acnB*	2.07		aconitate hydratase 2
PA1912		2.53	3.23	probable sigma-70 factor, ECF subfamily
PA2426	*pvdS*	5.42	10.43	sigma factor PvdS
PA2624	*idh*	5.28		isocitrate dehydrogenase
PA2640	*nuoE*	-4.48	-4.59	NADH dehydrogenase I chain E
PA2643	*nuoH*	-5.17	-4.35	NADH dehydrogenase I chain H
PA2646	*nuoK*	-3.44	-3.24	NADH dehydrogenase I chain K
PA2647	*nuoL*	-2.63	-2.53	NADH dehydrogenase I chain L
PA3407	*hasAp*	31.45	33.33	heme acquisition protein HasAp
PA3540	*algD*	3.96	3.90	GDP-mannose 6-dehydrogenase AlgD
PA3600	*rpl36*	434.78	1000.00	conserved hypothetical protein
PA3601	*ykgM*	232.56	175.44	conserved hypothetical protein
PA4063		29.07	24.75	probable ATP-binding component of ABC
PA4064		5.04	4.56	transporter
PA4065		3.92	3.97	hypothetical protein
PA4175	*piv*		5.62	protease IV
PA4370	*icmP*		2.92	Insulin-cleaving metalloproteinase outer membrane protein precursor
PA4834		6.45	6.07	hypothetical protein
PA4835		14.31	13.40	hypothetical protein
PA4836		22.42	20.24	hypothetical protein
PA4837		24.15	22.17	probable outer membrane protein precursor
PA4896		3.43	3.54	probable sigma-70 factor, ECF subfamily
PA5170	*arcD*		3.84	arginine/ornithine antiporter
PA5171	*arcA*		3.65	arginine deiminase
PA5172	*arcB*		3.60	ornithine carbamoyltransferase, catabolic
PA5173	*arcC*		4.14	carbamate kinase
PA5373	*betB*	4.85	3.87	betaine aldehyde dehydrogenase
PA5374	*betI*	28.01	7.17	Transcriptional regulator BetI
PA5499	*np20*	4.18	5.68	Transcriptional regulator np20
PA5500	*znuC*	2.39		zinc transport protein ZnuC

Ribosomal proteins and zinc

The genes PA3600 and PA3601 exhibited the highest upregulation (Table 4-2). These two genes are annotated as encoding "conserved hypothetical proteins" (Pseudomonas Genome Database at www.pseudomonas.com). Based on similarity searches, their products are predicted to be paralogues of the 50S ribosomal protein L36 encoded by *rpmJ* (PA4242) and the 50S ribosomal protein L31 encoded by *rpm*E (PA5049), respectively. Paralogues of proteins L36 are also present in *Vibrio cholerae* and *Neisseria meningitidis,* while paralogues of L31 are found in *Escherichia coli, Bacillus subtilis, Vibrio cholerae and Neisseria meningitidis* (Makarova *et al.,* 2001). The difference between them is that the first contains the metal-binding Zn-ribbon, which consists of four conserved cysteins, whereas the second is void of metal chelating residues. The Zn binding form is designated C+ and the paralogue C- (Makarova *et al.*, 2001). The genes PA3600 and PA3601 are C- forms. Detailed studies regarding the swap of these two proteins in ribosome structure have been performed on *Bacillus subtilis* and *Streptomyces coelicolor* (Nanamiya *et al.*, 2006, Owen *et al.*, 2007, Shin *et al.*, 2007). It was revealed that expression of C- form of ribosomal proteins L31 and L36 is connected with zinc limitation and regulated by the zinc uptake regulator *zur* (Owen *et al.,* 2007). Indeed, among the genes upregulated in the burn wound infection there was a *np20* gene (see PA5499, Table 4-2) which is homologous to *E. coli* gene *zur* encoding zinc uptake regulator and it has been reported to be involved in virulence (Gallagher *et al.,* 2002). In comparison with planktonic growth, gene *znu*C encoding a zinc transport protein was upregulated. It is interesting that the overexpression of genes PA3600 and PA3601 as well as *np20* has been observed when *P. aeruginosa* cells were in contact with eukaryotic tissue, e.g. in rat peritoneum *(Mashburn et al.,* 2005) or in the epithelial cell lines (Chugani & Greenberg, 2007).

Iron starvation regulated genes

One of the major bottlenecks that bacterial pathogens have to overcome is the availability of iron (Takase *et al.,* 2000, Letoffe *et al.,* 1998). In the transcription profile of burn wound infection, iron starvation genes are clearly upregulated. In this work, 50 of the 118 genes

that Ochsner *et al* (2002) reported to be involved in iron starvation, were differentially regulated (Table 4-3).

P. aeruginosa possesses two distinct endogenous siderophores, pyoverdine and pyochelin. The pyoverdin synthesis pathway (involving *pvd* genes) was upregulated in comparison to both controls. However, the pyochelin synthesis pathway was more expressed (involving *pch* genes) during planktonic growth. This was expected, as pyochelin is less efficient than pyoverdine, thus under iron limited conditions during host infection the siderophores are expressed in a hierarchy promoting a more efficient system. In addition to ferric iron provided by siderophores, *P. aeruginosa* acquires iron from heme and heme-containing proteins such as haemoglobin. In this work, the gene *hasAp* encoding the heme acquisition protein was overexpressed together with the gene *hemO* (*pigA*) encoding for heme oxygenase which degrades heme to biliverdine and releases iron.

Table 4-3: Expression values of those genes (previously reported to response to iron starvation by Ochsner *et al,*. 2002) that were differentially expressed in respect to the planktonic and biofilm growth in this work.

PA number	Gene	Fold change compared to: Planktonic	Biofilm	Product name
PA0472	*fiuI*		3.93	probable sigma-70 factor, ECF subfamily
PA0672	*hemO*		6.84	heme oxygenase
PA0707	*toxR*	4.34	4.16	transcriptional regulator ToxR
PA1134		3.67	3.69	hypothetical protein
PA1300			5.49	probable sigma-70 factor, ECF subfamily
PA1301			4.22	probable transmembrane sensor
PA2033			3.03	hypothetical protein
PA2034		-1.29	4.14	hypothetical protein
PA2383		2.69	4.24	probable transcriptional regulator
PA2384		3.09	12.29	hypothetical protein
PA2385	*pvdQ*	5.70	5.47	PvdQ
PA2386	*pvdA*	4.16	4.36	L-ornithine N5-oxygenase
PA2393		2.84	3.47	probable dipeptidase precursor
PA2395	*pvdO*	3.79	3.90	PvdO
PA2404		2.94	4.19	hypothetical protein
PA2405		4.01	4.63	hypothetical protein
PA2406		3.51		hypothetical protein
PA2407		2.57	2.50	probable adhesion protein
PA2411		6.05	15.46	probable thioesterase

PA2412		5.76	13.16	conserved hypothetical protein
PA2413	*pvdH*	3.69	4.69	L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase, PvdH
PA2424	*pvdL*	3.23	4.57	PvdL
PA2425	*pvdG*	2.34	3.78	PvdG
PA2426	*pvdS*	5.42	10.43	sigma factor PvdS
PA2531		2.33		probable aminotransferase
PA3397	*fpr*	2.93		ferredoxin—NADP+ reductase
PA3407	*hasAp*	31.45	33.33	heme acquisition protein HasAp
PA3530	*bfd*		2.67	conserved hypothetical protein
PA3811	*hscB*	2.32		heat shock protein HscB
PA3812	*iscA*	2.46		probable iron-binding protein IscA
PA4175	*piv*		5.62	protease IV
PA4220	*fptB*	-2.67	7.49	hypothetical protein
PA4221	*fptA*	-1.40	3.72	Fe(III)-pyochelin outer membrane receptor precursor
PA4223	*pchH*	-3.51	2.93	probable ATP-binding component of ABC transporter
PA4224	*pchG*	-3.41	3.83	pyochelin biosynthetic protein PchG
PA4227	*pchR*		5.21	transcriptional regulator PchR
PA4230	*pchB*	-1.31	4.32	salicylate biosynthesis protein PchB
PA4370	*icmP*		2.92	Insulin-cleaving metalloproteinase outer membrane protein precursor
PA4467			10.06	hypothetical protein
PA4468	*sodM*	3.57	16.95	superoxide dismutase
PA4469			9.95	hypothetical protein
PA4470	*fumC1*		15.70	fumarate hydratase
PA4471	*fagA*	3.22	7.59	hypothetical protein
PA4570			14.01	hypothetical protein
PA4833			5.77	conserved hypothetical protein
PA4896		3.43	3.54	probable sigma-70 factor, ECF subfamily
PA5150			3.58	probable short-chain dehydrogenase
PA5217			2.71	probable binding protein component of ABC iron transporter
PA5531	*tonB*		3.06	TonB protein

The iron starvation response also induces many factors directly involved in virulence. For example, the transcriptional regulator *toxR* (*regA*) controls the expression of Exotoxin A. There were also two proteolitic enzymes overexpressed: (i) the gene *icm*P encoding an insulin-cleaving metalloproteinase outer membrane protein precursor, which is capable of cleaving beta and alpha fibrinogen chains (Fricke *et al.*, 1999); and, (ii) the protease IV encoded by *piv* (also known as *prpL*) which is an endoprotease that cleaves iron containing

proteins and which has been reported to be necessary for corneal infection and contributes to persist in rat chronic pulmonary infection model (O'Callaghan *et al.*, 1996, Wilderman *et al.*, 2001). Interestingly, in the burn wound infection, there was no significant increase in expression of 2 other proteases well known to be involved in *P. aeruginosa* virulence in other infection conditions, namely, elastase and alkaline protease.

Uncharacterised gene clusters

There are many genes encoding hypothetical and conserved hypothetical proteins differentially expressed in all analyses done. The two predicted operons (using Prodoric database, prodoric.tu-bs.de), PA4063-PA4066 and PA4834-PA4837 are worth closer analysis. According to the Pseudomonas Genome Database, the gene PA4063 encodes a protein that is exported across the inner membrane (Lewanza *et al.,* 2005). According to database the genes PA4064 and PA4065 are predicted to be an ABC-type antimicrobial peptide transport system, ATPase and permease component, respectively. The last gene in this operon, PA4066, encodes a putative lipoprotein which has also been proven to be transported across the inner membrane (Lewenza *et al.*, 2005). It can be hypothesised that the operon PA4063-PA4066 encodes the antimicrobial transport system.

The gene PA4837 has a 45% aminoacid similarity to the ferrichrome iron receptor in *Enterobacter agglomerans.* Also, this gene is predicted to encode FhuE, an outer membrane receptor for ferric coprogen and ferric-rhodotorulic acid, while also being a TonB-dependent receptor. The following gene in the operon is PA4836, which encodes a hypothetical conserved protein with a conserved domain of nicotianamine synthase protein. This protein is involved in iron acquisition in plants. However, it is not clear what is exactly the product of this gene in *P. aeruginosa*. The gene PA4835 encodes for a hypothetical protein with at least one transmembrane helice. The last gene in the cluster, PA4834, is predicted to encode a putative permease. It is possible that the last 2 genes encode for the transporter of a novel siderophore encoded by PA4836 while the product of the first gene in this operon is responsible for the receptor sensing this siderophore.

Energy production and metabolism

When comparing the burn wound infection to the planktonic growth control, there was upregulation of the citrate cycle genes encoding for the proteins responsible for transformation of citrate to 2-oxoglutarate *acnB* (PA1787) and *idh* (PA2624). The genes *sdhC* and *sdhD*, encoding for succinate dehydrogenase C and D subunits were also overexpressed. Succinate dehydrogenase transforms succinate to fumarate and is one of the complexes included in oxidative phosphorylation. The other oxidative phosphorylation complexes, NADH dehydrogenase (PA2640, PA2643, PA2646 and PA2647) and Cytochrome C oxidase (PA0105-8) were all downregulated. When the burn wound infection is compared to the biofilm growth there was downregulation of all complexes of oxidative phosphorylation. All proteins from these systems contain both heme and iron, thus the conditions of iron starvation could explain the lower expression of genes encoding these proteins. Another interesting finding was the overexpression (in comparison with the biofilm growth) of the arginine deaminase pathway (involving the genes *arcABCD*), which may use arginine as a source of energy by fermentation during anaerobic conditions (Vander Wauven *et al.*, 1984). This finding raises the question of whether *P. aeruginosa* infecting burn wound has the anaerobic fermentation active parallel to the inhibited aerobic oxidation. That could be connected with the limited iron, which is needed for active oxidative phosphorylation complexes. This hypothesis will be further discussed in section 5.2. However, these different results may be also due to the heterogeneity of the cellular states in the samples, as they generally a mixture of bacteria at different depths in a biofilm, where steep oxygen gradients prevail. In general, it can be seen that *P. aeruginosa* residing at the burn wound surface have lower viability and are less metabolically active compared to the planktonic and biofilm growth on rich LB medium.

In comparison to both controls, the induction of the PA0102 gene encoding a probable carbonic anhydrase can be observed. This is a zinc containing enzyme that catalyzes the interconvertion of carbon dioxide and bicarbonate. Carbonic anhydrase has been reported to be involved in maintaining the pH in the cytoplasm of *Helicobacter pylori* colonising gastric

mucosa (Bury-Mone *et al.*, 2008). Also, the same gene is upregulated when *Salmonella enterica* serovar *Typhimurium* is taken up by macrophages (Valdivia & Falkow, 1997).

The gene *betB* encodes a betaine aldehyde dehydrogenase, which is regulated by the product of gene *betI.* Both of these genes were upregulated when comparing burn wound infection with the control conditions. Betaine aldehyde dehydrogenase is responsible for production of glycine betaine which is the major osmoprotectant for bacterial cells (Csonka & Hanson, 1991). There are also reports showing that *P. aeruginosa* is able to utilise phosphatydylocholine from lung surfactant as carbon and nitrogen sources. The first step in the transformation of this compound is the cleavage of phophatidylocholine by phospholipase C, which results in phophorylcholine transformed later with use of enzymes encoded by *bet* operon (Son *et al.,* 2007). However, only *betB* was shown to be overexpressed in this work.

4.2.1.2. Regulatory networks

The genome of *P. aeruginosa* encodes 5570 open reading frames. These genes are regulated via an extensive network of transcriptional regulators and two-component regulatory systems (Stover *et al.,* 2000). Various regulators are responsible for standard housekeeping reactions within the cell but also for virulence factors and survival during infection. Figure 4-2 presents a simplified interrelation between various regulators which were upregulated in the burn wound infection and the genes which were controlled by these regulators.

When comparing the burn wound infection to the *in vitro* planktonic growth there was an overexpression of the *vfr* gene encoding for the virulence factor regulator Vfr, which is a cAMP receptor protein and has been termed due to its effect on the production of several virulence factors (West *et al.*, 1994a). Regulation of these factors is principally due to the induction of the *las* QS system (Albus *et al.,* 1997), followed by subsequent induction of the *rhl* system by *las*. The Vfr regulator also directly controls ToxR, the regulator of Exotoxin A production and the synthesis of Protease IV (gene *piv*) (West et al., 1994a).

One of the regulators overexpressed during the burn wound infection (compared to the biofilm control) is AlgU. This is an alternative sigma (σ) factor also known as RpoE or σ^{22} belonging to the family of extracytoplasmic function (ECF) sigma factors. It has been shown to be important in converting from the nonmucoid to mucoid phenotype in CF patients (Schurr *et al.*, 1996), as well as in oxidative and heat-shock stress (Schurr & Deretic, 1997). AlgU induces the expression of *alg*D (PA3540) which is the first gene in the alginate biosynthesis operon *alg*D-A (PA3540-PA3551). The anti-sigma factor of AlgU is MucA encoded by the *mucA* gene, which was also upregulated in the burn wound infection. This underscores the precise regulation of alginate production in bacteria infecting the host (Schurr *et al.,* 1996). AlgR is another transcriptional regulator involved in alginate production, which was also upregulated during the burn wound infection. This member of the LytTR family of a two-component transcriptional regulator (Nikolskaya & Galperin, 2002) is also reported to be required for twitching motility utilizing type IV pili (Whitchurch *et al.*, 1996) and represses the production of hydrogen cyanide and the putative cbb_3-type cytochrome PA1557 (Lizewski *et al.*, 2004).

PvdS is another ECF sigma factor upregulated in the burn wound infection. Figure 4-2 presents the genes upregulated by PvdS. PvdS, together with anti-sigma factor FpvR (PA2388) regulates the production of pyoverdine and also controls the production of the different extracellular virulence factors, i.e. Protease IV and exotoxin A. While PvdS is controlled by a Fur repressor (ferric uptake regulator), the other genes controlled by Fur are shown in Figure 4-2.

In addition to PvdS and AlgU there are 19 ECF sigma factors present in the *P. aeruginosa* genome. Of these, 14 display sequence similarity with iron starvation sigma factors (Llamas *et al.*, 2008), while of these, 4 are induced during the burn wound infection: (i) PA0472 encoding ECF protein Fiu, which regulates iron uptake via ferrichrome (Llamas *et al.,* 2008); (ii) PA1300 with a similarity of 54% to *E. coli* FecI and probably regulates heme uptake (Llamas *et al.,* 2008); (iii) PA1912 encoding FemI ECF protein which regulates the uptake via heterologous siderphore mycobactin/carboxymycobactin, and (iv) PA4896 with a similarity of 64% to *E. coli* FecI and functions for siderophore uptake (Llamas *et al.,* 2008).

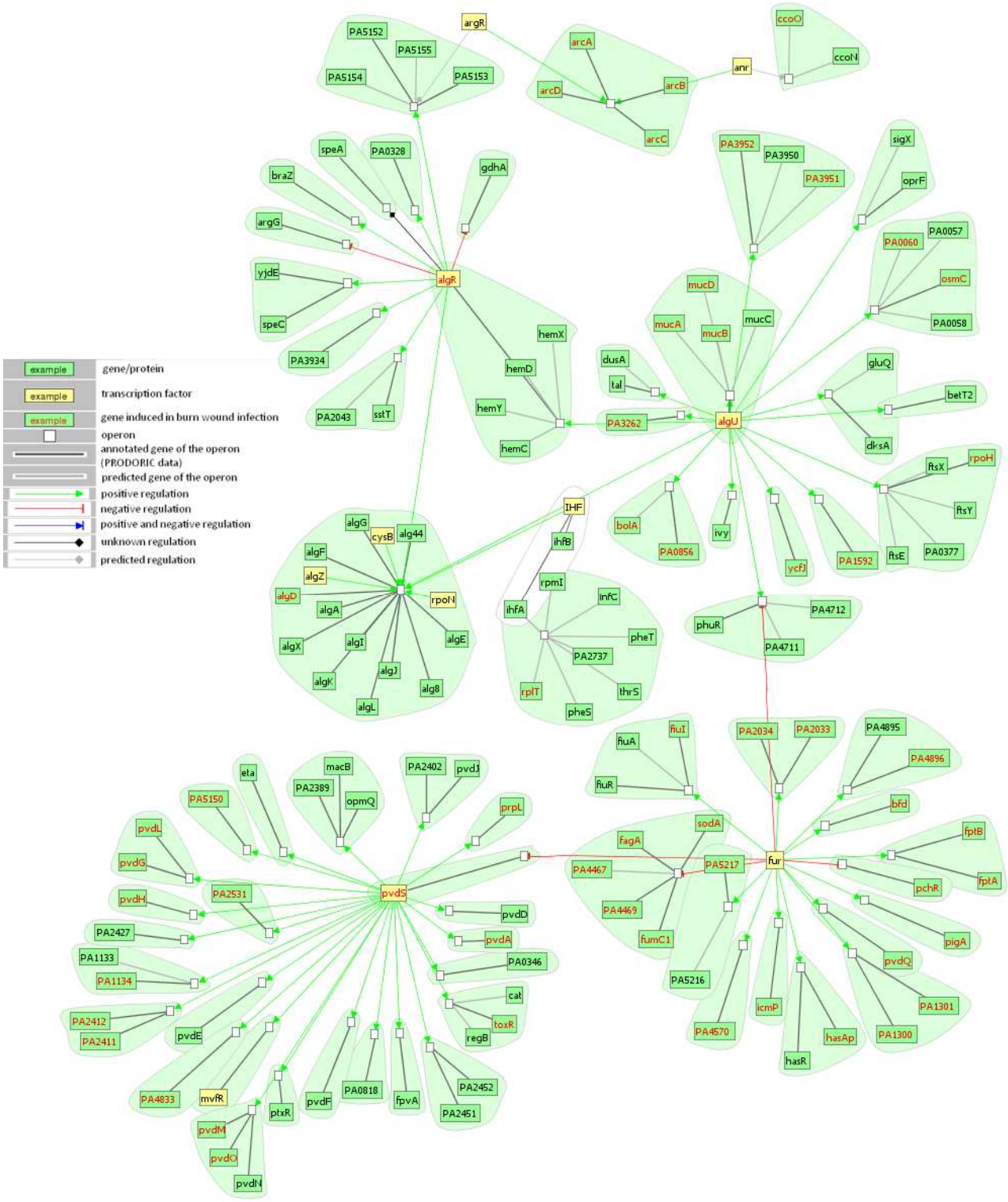


Figure 4-2: Graphic presentation of the regulatory relations in the burn wound infection. The network was created using the web based interface ProdoNet (Klein *et al.*, 2008). The legend was adapted from http://prodoric.tu-bs.de/prodonet/

4.2.2. Cystic Fibrosis pulmonary infection - a preliminary investigation

To assess the possible differences and similarities of *in vivo* gene expression of a CF isolate as compared to planktonic controls, transcription analysis was performed on a sputum sample obtained from a CF patient. The CF patient's sputum was treated in a similar manner to the burn wound samples. The *P. aeruginosa* strain isolated from the patient was cultivated in LB medium and the planktonic control performed as for the other clinical strains. The microarray data analysis was performed on the duplicates of the clinical sample and duplicates of the planktonic growth sample. The same conditions and the same pfp value threshold were set at 0.05. Table 4-4 presents the list of those differentially regulated genes.

Table 4-4: Differentially regulated genes (pfp < 0.05) during a CF pulmonary infection.

PA Number	Gene	Fold change	Product Name
PA0105	*coxB*	-94.13	cytochrome c oxidase, subunit II
PA0106	*coxA*	-353.90	cytochrome c oxidase, subunit I
PA0107		-274.10	conserved hypothetical protein
PA0108	*coIII*	-509.26	cytochrome c oxidase, subunit III
PA0110		-80.38	hypothetical protein
PA0111		-188.03	hypothetical protein
PA0176	*aer2*	-79.93	aerotaxis transducer Aer2
PA0179		-104.28	probable two-component response regulator
PA0465	*creD*	120.48	inner membrane protein CreD
PA0466		81.97	hypothetical protein
PA0578		82.64	conserved hypothetical protein
PA0672	*hemO*	142.86	heme oxygenase
PA0866	*aroP2*	-281.13	aromatic amino acid transport protein AroP2
PA0996	*pqsA*	-717.41	probable coenzyme A ligase
PA0997	*pqsB*	-771.42	Homologous to beta-keto-acyl-acyl-carrier protein synthase
PA0998	*pqsC*	-1039.03	Homologous to beta-keto-acyl-acyl-carrier protein synthase
PA0999	*pqsD*	-300.62	3-oxoacyl-[acyl-carrier-protein] synthase III
PA1000	*pqsE*	-587.49	Quinolone signal response protein
PA1001	*phnA*	-331.43	anthranilate synthase component I
PA1002	*phnB*	-184.82	anthranilate synthase component II
PA1027	*pcd*	-72.66	probable aldehyde dehydrogenase
PA1173	*napB*	-70.45	cytochrome c-type protein NapB precursor
PA1174	*napA*	-195.49	periplasmic nitrate reductase protein NapA
PA1176	*napF*	-73.36	ferredoxin protein NapF
PA1183	*dctA*	77.52	C4-dicarboxylate transport protein
PA1300		88.50	probable sigma-70 factor, ECF subfamily

PA1922	*feuA*	100.00	probable TonB-dependent receptor
PA1924		74.07	hypothetical protein
PA1925		185.19	hypothetical protein
PA1927	*metE*	263.16	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase
PA2365		-190.18	conserved hypothetical protein
PA2366		-114.98	conserved hypothetical protein
PA2368		-87.25	hypothetical protein
PA2386	*pvdA*	66.23	L-ornithine N5-oxygenase
PA2397	*pvdE*	59.17	pyoverdine biosynthesis protein PvdE
PA2398	*fpvA*	312.50	ferripyoverdine receptor
PA2407		101.01	probable adhesion protein
PA2409		46.73	probable permease of ABC transporter
PA2426	*pvdS*	88.50	sigma factor PvdS
PA2762		-140.54	hypothetical protein
PA2840	*deaD*	54.35	probable ATP-dependent RNA helicase
PA2911		51.02	probable TonB-dependent receptor
PA2937		-85.07	hypothetical protein
PA3236		-103.34	probable glycine betaine-binding protein precursor
PA3281		52.08	hypothetical protein
PA3407	*hasAp*	666.67	heme acquisition protein HasAp
PA3416		-84.51	probable pyruvate dehydrogenase E1 component, beta chain
PA3431	*ywbG*	79.37	conserved hypothetical protein
PA3432		119.05	hypothetical protein
PA3600	*rpl36*	1428.57	conserved hypothetical protein
PA3601	*ykgM*	263.16	conserved hypothetical protein
PA3922		-255.59	conserved hypothetical protein
PA3923		-138.47	hypothetical protein
PA4063		121.95	hypothetical protein
PA4064		92.59	probable ATP-binding component of ABC transporter
PA4296	*pprB*	-84.09	two-component response regulator PprB
PA4467		54.95	hypothetical protein
PA4470	*fumC1*	63.69	fumarate hydratase
PA4834		71.43	hypothetical protein
PA4835		91.74	hypothetical protein
PA4836		88.50	hypothetical protein
PA4837		66.23	probable outer membrane protein precursor
PA4838		99.01	hypothetical protein
PA4913		-73.95	probable binding protein component of ABC transporter
PA5373	*betB*	57.14	betaine aldehyde dehydrogenase
PA5374	*betI*	285.71	transcriptional regulator BetI
PA5396		-96.75	hypothetical protein
PA5410	*gbcA*	-125.86	GbcA

PA5504		59.17	probable permease of ABC transporter
PA5535		51.81	conserved hypothetical protein
PA5540		69.93	hypothetical protein
PA5541	*pyrQ*	85.47	dihydroorotase

The most striking result was the similarity of gene expression to the burn wound infection. More specifically, ribosomal proteins PA3600 and PA3601 were among the most upregulated in both infections (see Tables 4-2 and 4-4). There were also two other genes, PA4063 and PA4064, which encode a putative excretion system, as well as the whole operon of the putative novel siderophore system PA4834-38 described above. There are two genes which were reported to be involved in this process, *betB* and the regulator *betI*. The gene *betB* encodes betaine aldehyde dehydrogenase. It is important to mention that these two genes were upregulated as well in the burn wound infection. In addition, these genes have been reported to be involved in utilisation of the phosphatydylocholine as a carbon and nitrogen source for *P. aeruginosa* infecting CF patients lungs (Son *et al.,* 2007).

Another feature proven to be crucial for infection is iron acquisition. In the CF patient infection, there was overexpression of PvdS sigma factor together with genes responsible for pyoverdin synthesis and its receptor FpvA. There was also an overexpression of the probable sigma factor PA1300 as well as the putative TonB-dependent receptors PA1922 and PA2911. Again, similarly to the burn wound infection, there was high overexpression of *hasAp*, which encodes for a heme acquisition protein.

PQS system was downregulated in comparison to planktonic growth. A significant decrease was observed over the whole PQS synthesis system *pqsA-E* and *phnAB* (PA0996-PA1002). This downregulation could be explained by the elevated concentrations of PQS in the lung of CF patient with persistent chronic infection (pers. com. Dr. Susanne Haeussler). Also, the response regulator PprB belonging to the PprA-PprB two-component system was downregulated. This two-component system has been shown to modulate a broad range of QS-related genes in *P. aeruginosa* including those coding for PQS (Dong *et al.,* 2005). The current list of differentially regulated genes shows similarities to the burn wound infection.

Genes PA3600, PA4835, *pqsA* and *hasAp* are further compared across all infection conditions later in this chapter (see Figure 4-13). To further elucidate the gene expression of CF patient infection additional samples will need to be collected.

4.3. *P. aeruginosa* infection models

Infection models are important tools used to elucidate bacterial pathogenesis. Model are crucial to test novel hypotheses pertaining mechanisms underlying virulence, potential targets, the mode of action of anti-bacterial compounds and to screen possible new compounds or intervention strategies. In this work, both the plant infection model using lettuce leaves and the novel mouse tumour infection model were evaluated. It is proposed that the mouse tumour infection model can mimic the chronic infection of *P. aeruginosa*, which is a state of infection persistent in CF patients, as well as the long lasting infection of burn wounds. Firstly, the lettuce infection was tested for its resemblance of a real infection at the transcriptomic level.

4.3.1. Lettuce infection model

4.3.1.1. Lettuce infection

Since *P. aeruginosa* is both a mammalian and a plant pathogen (Mahajan-Miklos *et al.*, 2000, Rahme *et al.*, 2000), the lettuce leaf infection model has been used to evaluate a number of aspects of pathogenicity by *P. aeruginosa*, namely the hierarchy of QS and virulence factors, as well as anaerobic growth (Aendekerk *et al.*, 2005, Filiatrault *et al.,* 2006, Wagner *et al.*, 2007). In the current work, the aim was to perform global expression profiling of *P. aeruginosa* infecting the lettuce to assess the potential usefulness of the lettuce as a model for mammalian infection. First, the level of infection of *P. aeruginosa* isolates PBCLOp10, 11, 17 and *P. putida* KT2440 was measured. *P. putida* KT2440 was used as a non-infection control. Figure 4-3 shows the visual deterioration of the lettuce leaf after injection of different *Pseudomonas* strains at both day 0 and day 5. For the three *P. aeruginosa* clinical strains the necrosis and tissue maceration was visually similar after 5 days. *P. putida* KT2440 strain did not produce such a marked change in the lettuce leaf as in comparison to those clinical strains.

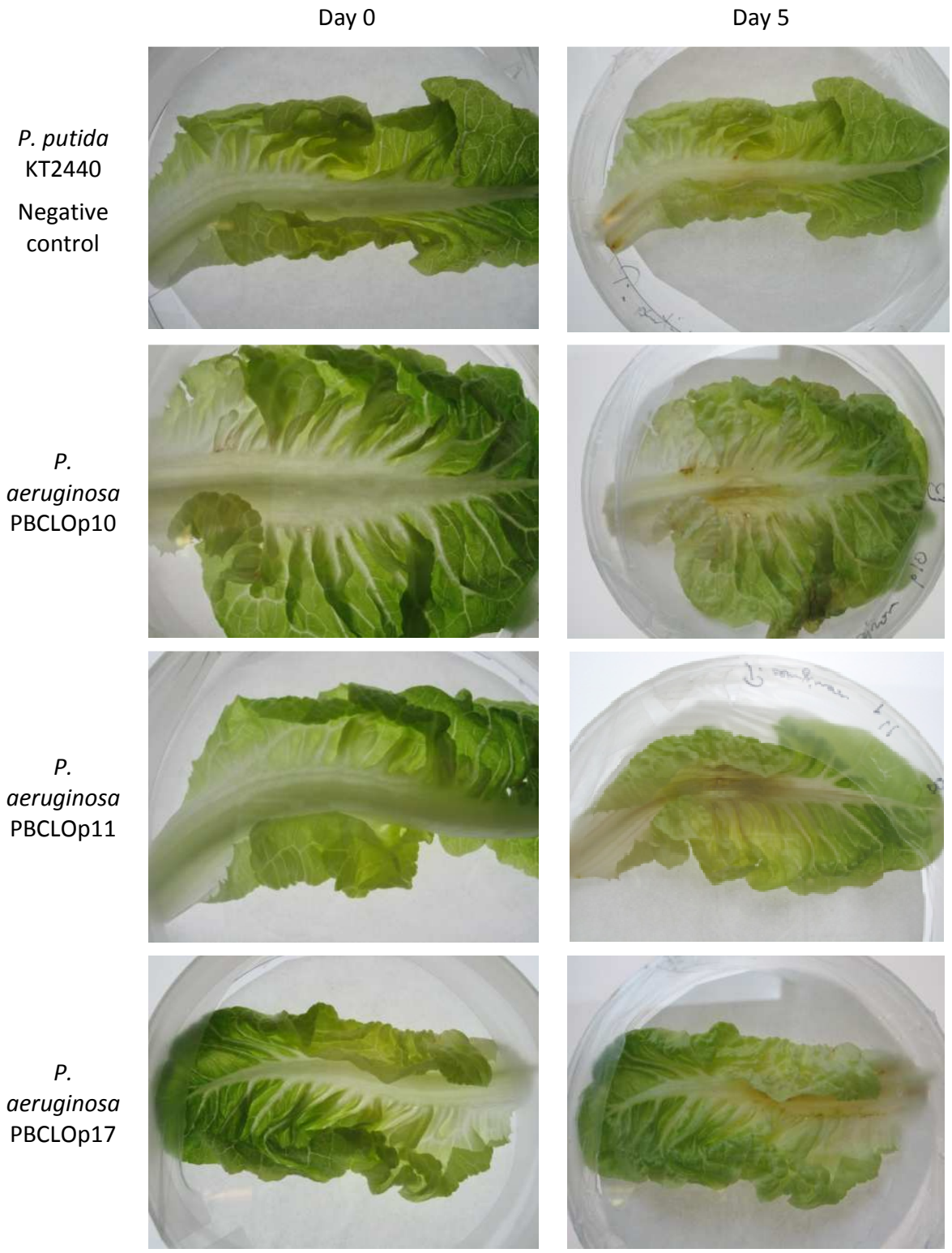


Figure 4-3: Infection of lettuce mid-ribs by *P. putida* (as a non-infection control) and the 3 *P. aeruginosa* clinical strains PBCLOp10, 11 and 17 at days 0 and 5.

4.3.1.2. Transcription profiling of *P. aeruginosa* infecting lettuce

Table 8-3 on page 177 shows all of the expression values obtained from comparison of the lettuce infection model with the planktonic and biofilm growth controls. Table 4-5 summarises the expression levels of the genes discussed in this chapter.

Table 4-5: Summary of genes from table 8-3 and those that are discussed in this section.

PA Number	Gene	Fold change compared to Planktonic	Biofilm	Product Name
PA0280	*cysA*	6.63	6.75	sulfate transport protein CysA
PA0281	*cysW*	6.80	5.95	sulfate transport protein CysW
PA0282	*cysT*	3.37	2.72	sulfate transport protein CysT
PA0283	*sbp*	8.56	8.41	sulfate-binding protein precursor
PA0298	*spuB*	2.94	2.46	probable glutamine synthetase
PA0472	*fiuI*	2.43	9.38	probable sigma-70 factor, ECF subfamily
PA0520	*nirQ*		2.64	regulatory protein NirQ
PA0929	*pirR*	3.14	6.63	two-component response regulator
PA1176	*napF*	-3.83	3.26	ferredoxin protein NapF
PA1177	*napE*		26.32	periplasmic nitrate reductase protein NapE
PA1544	*anr*	2.32		transcriptional regulator anr
PA1779	*nasA*	3.59	3.51	assimilatory nitrate reductase
PA1780	*nirD*	7.32	5.99	assimilatory nitrite reductase small subunit
PA1781	*nirB*	3.86	3.91	assimilatory nitrite reductase large subunit
PA1783	*nasA*	4.05	4.02	nitrate transporter
PA1838	*cysI*	3.46	2.85	sulfite reductase
PA2426	*pvdS*	1.79	3.45	sigma factor PvdS
PA2570	*lecA*	3.18	5.66	LecA
PA2664	*fhp*	42.02	42.92	flavohemoprotein
PA2718			2.44	probable transcriptional regulator
PA2847		312.50	312.50	conserved hypothetical protein
PA3479	*rhlA*	4.34		rhamnosyltransferase chain A
PA3899		2.08	3.69	probable sigma-70 factor, ECF subfamily
PA3914	*moeA1*	2.71	2.68	molybdenum cofactor biosynthetic protein A1
PA3915	*moaB1*	7.01	5.88	molybdopterin biosynthetic protein B1
PA3916	*moaE*	2.70	3.73	molybdopterin converting factor, large subunit
PA3918	*moaC*	4.39	4.47	molybdopterin biosynthetic protein C
PA4442	*cysN*	2.27	3.27	ATP sulfurylase GTP-binding subunit/APS kinase
PA4443	*cysD*	3.94	3.64	ATP sulfurylase small subunit
PA5240	*trxA*	3.80		thioredoxin
PA5374	*betI*	13.48	3.45	transcriptional regulator BetI

The most upregulated gene during the infection of the lettuce was the PA2847 gene. According to the Pseudomonas Genome Database it encodes a conserved hypothetical protein, which has 8 predicted transmembrane helices and one domain of unknown function. The main features of the results of expression profiling in lettuce are addressed below.

Anaerobic/aerobic respiration

The gene *anr* encoding the anaerobic transcriptional regulator Anr was overexpressed in comparison with the planktonic control. Anr has been previously shown to be involved in the lettuce infection (Filiatrault *et al.,* 2006). However, the border between aerobic and anaerobic respiration in the lettuce infection is not clear when analysing the transcriptomic data. In comparison to the biofilm control, the genes *napF* and *napE* encoding the ferredoxin protein NapF and the periplasmic nitrate reductase protein NapE were upregulated, while in comparison to the planktonic control, the whole *nap* system was downregulated. The *nirQ* gene has been shown to be essential for anaerobic growth in the lettuce infection (Filiatrault *et al.,* 2006) and it was induced in this work. On the other hand, there was a clear induction of the assimilatory nitrate reductase (involving genes *nasC*, *nirD*, *nirB* and the transporter encoding gene *nasA*). These are responsible for nitrate reduction to NH_3, which is used for L-glutamine synthesis (PA0298 encoding for a probable glutamine synthase and was induced in the lettuce infection). Assimilatory reduction is not used for anaerobic respiration. Moreover, there was a strong overexpression of the *fhp* gene encoding flavohemoprotein, which has been reported to be involved in detoxification of nitric oxide under aerobic conditions. Together with nitrate reductase there was overexpression of molybdopterin, which acts as a cofactor for this enzyme. These genes were *moeA1*, *moeB1*, *moaE* and *moaC.*

Quorum sensing

The lettuce infection model has been also used to assess the hierarchy of the QS system in *P. aeruginosa* (Wagner *et al.,* 2007). In the current work, there was an overexpression of the *lasI* gene encoding autoinducer synthesis protein LasI from the *las* QS system, (in comparison with biofilm growth). However, in comparison to planktonic growth, the genes directly

regulated by the *rhl* system: *rhlA* encoding for rahmnosyltransferase chain A and *lecA* coding for PA-I galactophilic lectin were upregulated. In comparison to the biofilm control the induction of a probable transcriptional regulator PA2718 was observed. It has been shown that the strain with mutation of this gene was unable to cause severe infection of the lettuce leaf (Wagner *et al.,* 2007). Additionally, according to the Prodoric database, there were 50 genes induced and 27 repressed, which have been previously known to be QS regulated (Schuster *et al.,* 2003) in comparison with biofilm growth and only 10 upregulated and 67 genes downregulated in comparison to the planktonic growth (Schuster *et al.,* 2003).

Sulfate reduction

The pathway overexpressed during the lettuce infection and not in the other infection conditions was a sulfate reduction starting with the transport system, ABC transporter compounds *cysA*, *cysW*, *cysT* encoding sulfate transport proteins and *sbp* encoding sulfate-binding protein precursor, finishing with those genes responsible for sulfate reduction: *cysD*, sulfate adenylyltransferase subunit 2, which condenses sulfate and adenosine 5'-triphosphate (ATP) to form adenosine 5'-phosphosulfate (APS); *cysN*, bifunctional sulfate adenylyltransferase subunit 1 / adenylylsulfate kinase protein transforming APS to 3'-phosphoadenosine 5'-phosphosulfate (PAPS); *cysI,* sulfite reductase reducing sulfite to H_2S; and *cysK*, cysteine synthase A involved in cysteine synthesis from H_2S and O3-acetyl-L-serine incorporating in this way acquired sulfur into metabolism of the bacterial cell (Hummerjohann *et al.*, 1998). In the whole pathway of sulfate reduction there is only one enzyme missing in this expression data, *cysH,* PAPS reductase which forms sulfite from PAPS. Efficient reduction of sulfate requires protein reductant thioredoxin (Chartron *et al.*, 2006), hence the overexpression of the *trxA* gene encoding this protein.

Iron starvation

Up-regulation of the sigma factor PvdS was reported in *in vivo* infection of the burn wound and the CF patients' infection and was seen in the plant infection with *P. aeruginosa*. This suggests that during the lettuce infection, iron is at its limiting concentration. Most of the genes regulated by PvdS do not show differential expression with any of the controls.

However, further regulation of iron starvation is present as there are genes upregulated such as: *pirR,* a probable two-component response regulator (PA0929), PA3899, PA0472 and PA1912 all encoding a probable sigma-70 factor, ECF family responsible for iron uptake. All of these regulators are induced in comparison with both planktonic and biofilm growth. Additionally, in comparison to the biofilm control the ECF sigma factor PA2468 (*foxI*) was induced.

4.3.2. Tumour mouse infection model

In order to assess the mouse tumour model as a possible real *in vivo*-like model of *P. aeruginosa* infection, three steps were taken. First, infection with the type strain *P .aeruginosa* PAO1 in comparison with the non-infectious *Pseudomonas putida* KT2440 strain was evaluated. Second, transcription profiling of three clinical strains used in the current study (*P. aeruginosa* PBCLO10, 11 and 17) was performed and finally, the comparison of another sequenced strain *P. aeruginosa* PA14 and its mutants was done.

4.3.2.1. *Pseudomonas* species in the tumour

The *P. putida* KT2440 *and P. aeruginosa* PAO1 were transformed with plasmid pMC5-lite carriyng the whole *luxCDABE* operon from *Photorhabdus luminescens* (Winson *et al.*, 1998). The *lux* is placed under the constitutive promoter. The advantage of the Lux operon is that it does not require any additional substrates to be given to the mice before measurement. The results derived from this experiment showed that *P. putida* KT2440 was not able to spread into the mouse's body and also did not colonise the tumour (Fig 4-4a). Therefore, it was considered a good negative control. The *P. aeruginosa* PAO1 was highly virulent towards the mouse and after 48 hours following inoculation three of the five mice died. Nevertheless, the initial systemic spread and subsequent colonisation of the tumour can be clearly seen (Fig 4-4b).

In addition, there was some evidence that *P. aeruginosa* can spread and colonise the gall bladder (Figures 4-4 and 4-6). In particular, *P. aeruginosa* PAO1 can be clearly observed to infect the gall blader of mice 2 and 3 at 24 h p.i. and mouse 1 at 48 h p.i. (Figure 4-4b), whereas *P. aeruginosa* PBCLOp10 infected the gall bladder of mice 1 and 5 at 48 h p.i. (Figure 4-6e). Such a result has yet to be published and warrants further investigation, however while interesting is beyond the scope of this work here.

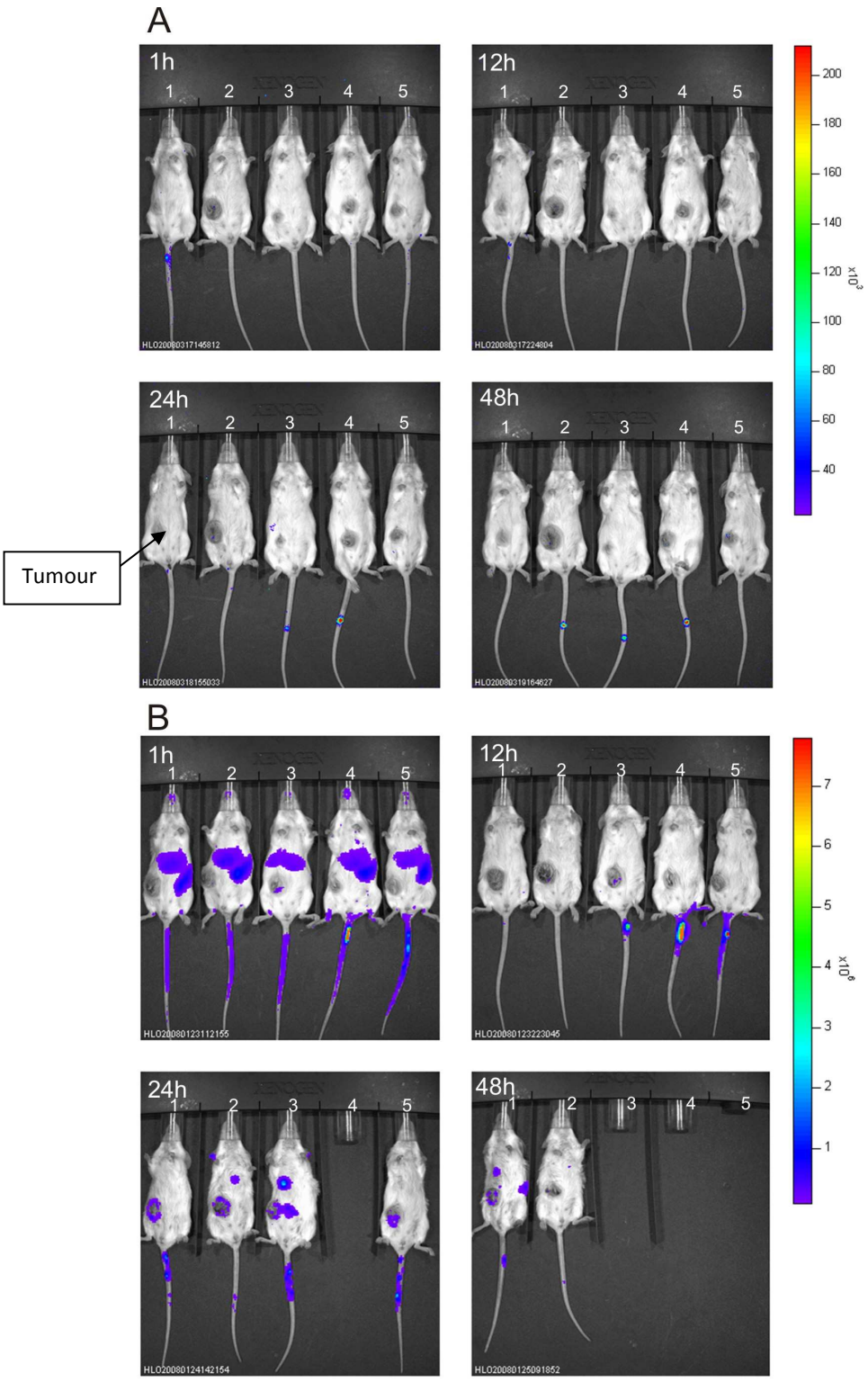


Figure 4-4: A) *P. putida* KT2440 bioluminescence with pBBRMCS5-LITE at 1 h, 12 h, 24 h, 48 h, B) *P. aeruginosa* PAO1 with pBBRMCS5-LITE at 1 h, 12 h, 24 h, 48 h.

4.3.2.2. Survival of *P. aeruginosa* clinical strains in mouse organs

Figure 4-5 shows the number of colonies obtained from the tumour compared to the spleen and liver after 48 hours p.i. All 3 strains were 2-3 orders of magnitude greater in the tumour compared to the liver and 4-5 orders of magnitude greater than in the spleen.

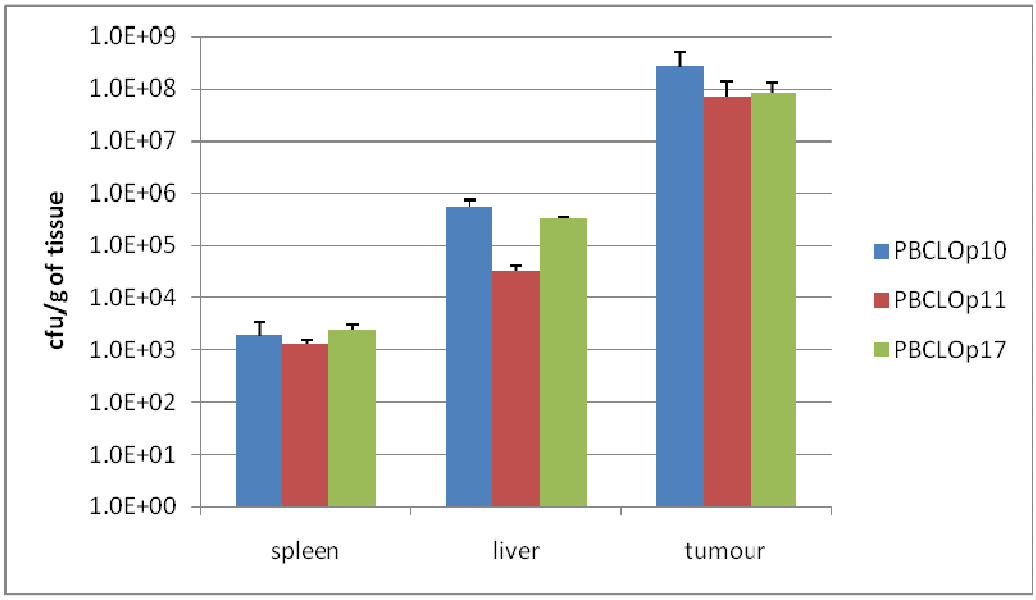


Figure 4-5: *P. aeruginosa* clinical strains in mice organs 3 days p.i.

In order to observe the *P. aeruginosa* tumour infection in real time, the plasmid pHL320, carrying the luciferase gene with the constitutive promoter was introduced into the strain PBCLOp10. Figure 4-6 represents the signal from *P. aeruginosa* PBCLOp10 cells 3, 12, 24, 36 and 48 hours p.i. In the first hours bacterial cells are present in the liver and in the spleen. Later, during the infection, bacterial cells were cleared from the mouse's organs, and then clearly present in the tumour at 24 h p.i. and remained there with slight fluctuations over 36 and 48 h p.i. (Figure 4-6).

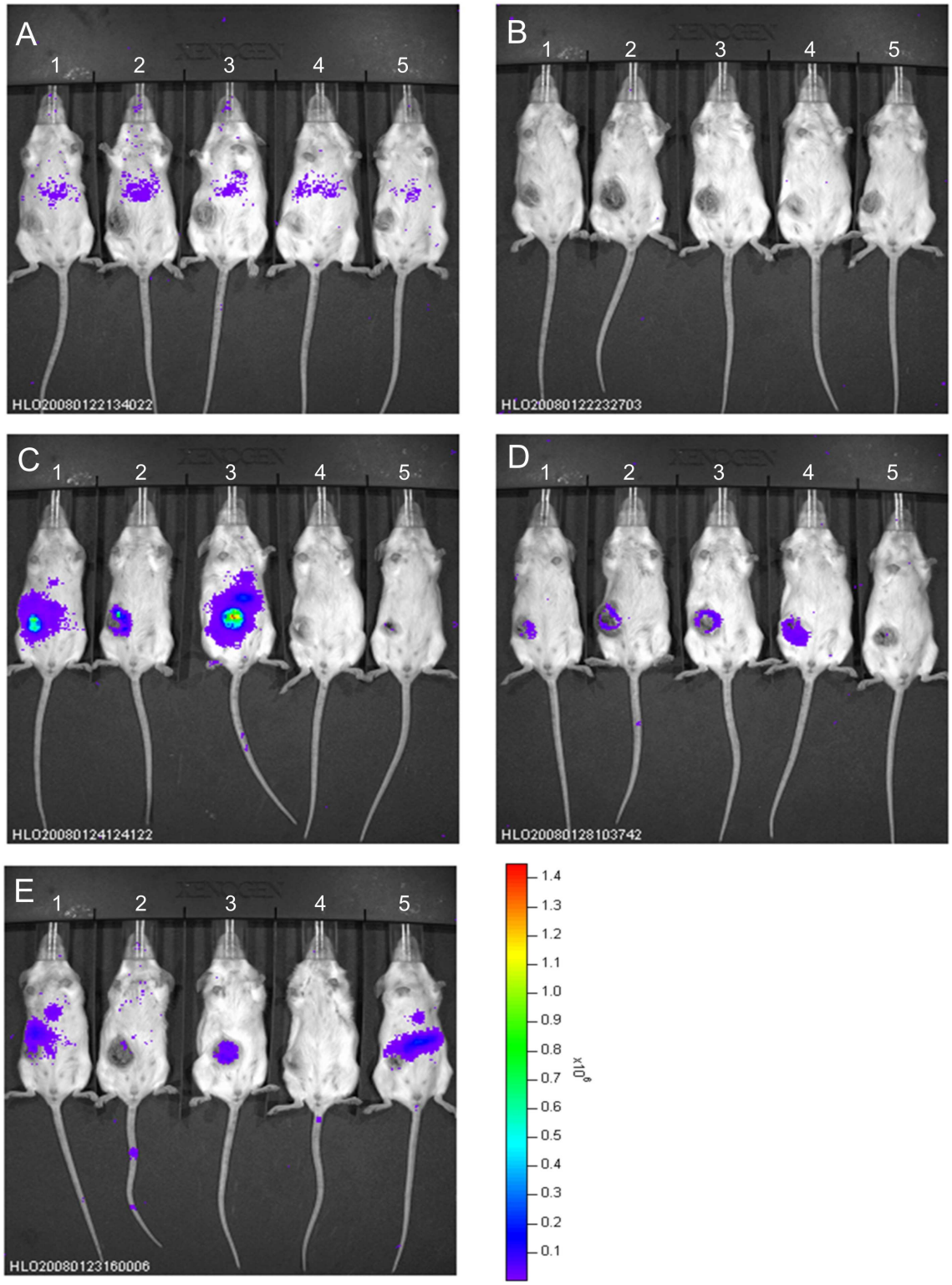


Figure 4-6: Bioluminescence of PBCLOp10 with pHL320 reporter plasmid. A) 3 h p. i.; B) 12 h p. i.; C) 24 h p. i.; D) 36h p. i.; E) 48 h p. i.

4.3.2.3. Localisation and persistence

The CT26 tumour is structured as a ring of the tumour cells with necrotic tissue inside. In the outer layer of the tumour, there is a rim of proliferating cells, while deeper in the quiescent cells and then in the centre, the necrotic tissue which is deprived from nutrients and oxygen (Sutherland, 1988). The localisation of the infection of *P. aeruginosa* was assessed by immune histology. As can be seen in Figure 4-7 viable bacterial cells were found in the ring. Low amounts of bacterial cells were found to penetrate the deeper layers into the quiescent cells or at the border with necrotic tissue. This observation makes the tumour infection very promising as a model, as microaerophilic conditions are believed to occur in the later stages of *P. aeruginosa* pulmonary infections in CF patients (Worlitzsch *et al.,* 2002).

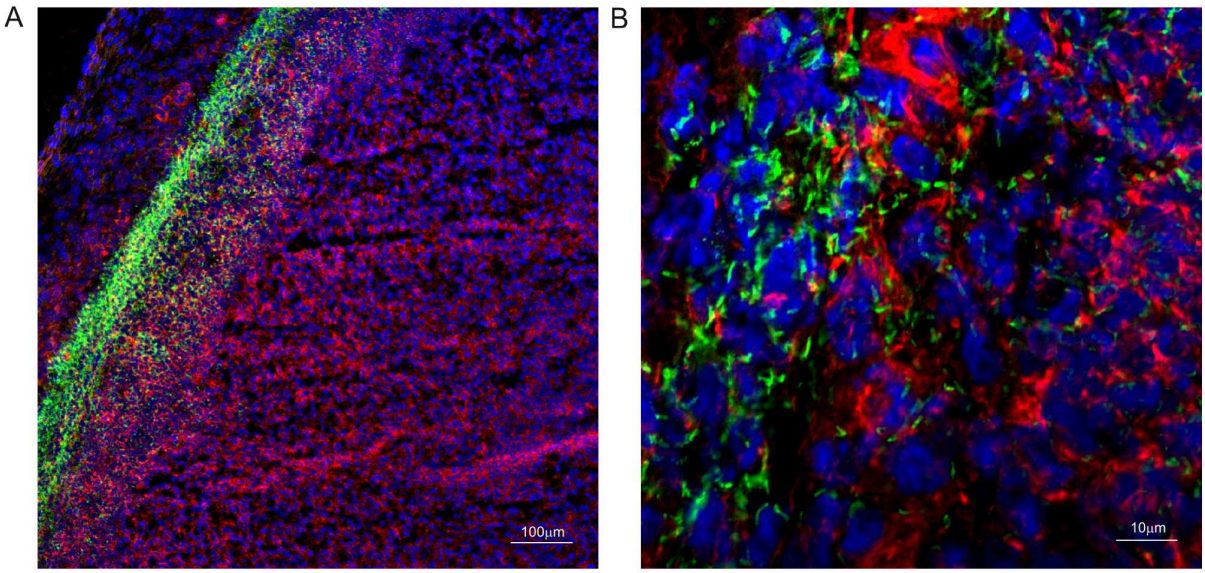


Figure 4-7: Histology of tumours colonised by *P. aeruginosa* PBCLOp10. A) magnification 10x, B) magnification 100x. Green colour – *P. aeruginosa* cells, blue – DNA, red – Cytoplasm.

4.3.2.4. Evaluating the mouse tumour model

4.3.2.4.1. Transcription profiling upon infection of tumour

In order to obtain an insight into the behaviour of *P. aeruginosa* colonising the tumour, the transcription profiling of the three clinical strains used in this study was performed. The complete results of differentially expressed genes are given in the Appendix Table 8-2 on page 164. Table 4-6 provides a summarised version of the genes discussed in this section.

Table 4-6: Summary of the genes discussed in the section 4.3.2.

PA Number	Gene	Fold change compared to Planktonic	Biofilm	Product Name
PA0044	*exoT*	6.51	10.52	exoenzyme T
PA0510	*nirE*	4.12	3.24	probable uroporphyrin-III c-methyltransferase
PA0515	*nirD*	4.63		probable transcriptional regulator
PA0517	*nirC*	3.53		probable c-type cytochrome precursor
PA0518	*nirM*	13.97	7.77	cytochrome c-551 precursor
PA0519	*nirS*	6.35	8.83	nitrite reductase precursor
PA0520	*nirQ*		4.14	regulatory protein NirQ
PA0836	*ackA*	3.32	5.03	acetate kinase
PA1580	*gltA*		-3.86	citrate synthase
PA1692	*pscS*	2.46		probable translocation protein in type III secretion
PA1695	*pscP*	2.93	2.81	translocation protein in type III secretion
PA1696	*pscO*	3.22		translocation protein in type III secretion
PA1699	*pcr1*	4.04		conserved hypothetical protein in type III secretion
PA1700	*pcr2*	8.82	5.61	conserved hypothetical protein in type III secretion
PA1701	*pcr3*	5.95		conserved hypothetical protein in type III secretion
PA1704	*pcrR*	2.08	3.11	transcriptional regulator protein PcrR
PA1706	*pcrV*	6.69	8.19	type III secretion protein PcrV
PA1707	*pcrH*	7.03	7.28	regulatory protein PcrH
PA1708	*popB*	5.57	7.43	translocator protein PopB
PA1709	*popD*	5.75	7.14	Translocator outer membrane protein PopD precursor
PA1711	*exsE*	4.26	5.81	ExsE
PA1712	*exsB*	2.76		exoenzyme S synthesis protein B
PA1714	*exsD*	5.52		ExsD
PA1715	*pscB*	7.81	4.80	type III export apparatus protein
PA1716	*pscC*	4.98		Type III secretion outer membrane protein PscC precursor
PA1717	*pscD*	5.64	5.47	type III export protein PscD
PA1718	*pscE*	11.83	6.06	type III export protein PscE

PA1719	*pscF*	4.32	2.96	type III export protein PscF
PA1720	*pscG*	3.94	3.58	type III export protein PscG
PA1721	*pscH*	4.13	4.14	type III export protein PscH
PA1722	*pscI*	4.61	4.38	type III export protein PscI
PA1723	*pscJ*	2.59		type III export protein PscJ
PA1724	*pscK*	2.41		type III export protein PscK
PA2128	*cupA1*	16.61	10.25	fimbrial subunit CupA1
PA2191	*exoY*	4.19	4.18	adenylate cyclase ExoY
PA3147	*wbpJ*	3.05		probable glycosyl transferase WbpJ
PA3148	*wbpI*	5.42	3.49	probable UDP-N-acetylglucosamine 2-epimerase WbpI
PA3149	*wbpH*	3.30		probable glycosyltransferase WbpH
PA3150	*wbpG*	4.08	4.51	LPS biosynthesis protein WbpG
PA3153	*wzx*	2.69		O-antigen translocase
PA3154	*wzy*	4.13	2.61	B-band O-antigen polymerase
PA3155	*wbpE*	4.08		probable aminotransferase WbpE
PA3156	*wbpD*	3.08		probable acetyltransferase WbpD
PA3158	*wbpB*	2.68		probable oxidoreductase WpbB
PA3159	*wbpA*	2.45		probable UDP-glucose/GDP-mannose dehydrogenase WbpA
PA3160	*wzz*	3.13		O-antigen chain length regulator
PA3309	*uspK*	3.31	8.31	conserved hypothetical protein
PA3407	*hasAp*	13.89	14.73	heme acquisition protein HasAp
PA3415			2.62	probable dihydrolipoamide acetyltransferase
PA3417			4.84	probable pyruvate dehydrogenase E1 component, alpha subunit
PA3476	*rhlI*	-4.14	-4.52	autoinducer synthesis protein RhlI
PA3477	*rhlR*	-9.43	-4.84	transcriptional regulator RhlR
PA3600	*rpl36*	17.30	37.88	conserved hypothetical protein
PA3601	*ykgM*	13.16	10.03	conserved hypothetical protein
PA3841	*exoS*	7.44	16.13	exoenzyme S
PA4352		7.02	22.99	conserved hypothetical protein
PA5170	*arcD*		14.77	arginine/ornithine antiporter
PA5171	*arcA*	6.64	15.85	arginine deiminase
PA5172	*arcB*	4.39	16.37	ornithine carbamoyltransferase, catabolic
PA5173	*arc*		8.22	carbamate kinase
PA5373	*betB*	4.91	3.92	betaine aldehyde dehydrogenase
PA5374	*betI*	9.91	2.53	transcriptional regulator BetI
PA5435	*oadA*		-3.05	probable transcarboxylase subunit

Type III secretion system

When comparing the expression values of the tumour model to the burn wound infection, the CF patient infection and the lettuce leaf infection the most striking difference was the upregulation of the type III secretion system (T3SS). The upregulated operons were *pscSPO, pcr123R, exsD-pscDEFGHIJK* responsible for secretion apparatus, *pcrVH-popBD* involved in protein translocation and the regulators *exsEB*. The genes encoding exoenzymes secreted via the T3SS were also overexpressed: (i) exoenzymes ExoT and ExoS, which are ADP-ribosyltransferases inhibiting phagocytosis by disrupting actin cytoskeletal rearrangement, focal adhesisns and signal transduction cascades important in phagocytic function and (ii) ExoY, a cytotoxin with adenylate cyclase activity.

O-antigen

Another interesting observation was the upregulation of most of the genes belonging to the cluster responsible for the B-band O-antigen. In comparison to the planktonic and biofilm controls, the induction of genes *wzz*, *wbpABDE* (only versus planktonic), *wzx*, *wzy* and *wbpGHIJ* (versus planktonic and biofilm) were upregulated. However, there was no significant difference in regulation of the A form of the O-antigen. *P. aeruginosa* possesses two of these forms, which are attached to core lipid A of Lipopolisaccharide (LPS) (Rocchetta *et al.*, 1999). Perhaps it is that the A form of the O-antigen is not present in these clinical strains or that they prefer to express only the B-band of the O-antigen. This fact requires further elucidation.

Biofilm

The *cupA1* (PA2128) gene belonging to the operon *cupA123* was also upregulated. This operon encodes the different components and assembly factors of a putative fimbrial structure required for biofilm formation (Vallet *et al.*, 2001). The ability to form biofilms is one of the factors for successful infection by *P. aeruginosa* (Singh *et al.,* 2000). Furthermore, the expression of the *cupA1* gene was linked to anaerobiosis (Vallet-Gely *et al.*, 2007).

Anaerobic respiration

Analysis of the energy production in *P. aeruginosa* infecting mice tumours revealed that they grew anaerobically. The genes responsible for nitrite reduction, *nirSMCDE* and regulator *nirQ* were overexpressed. Other expressed genes indicating anaerobic growth were the encoding enzymes involved in pyruvate fermentation. In comparison to the biofilm control there was induction of the genes PA3417 and PA3415 responsible for conversion of pyruvate to acetyl-CoA and in comparison to both controls gene *ackA* (PA0836) encoding acetate kinase. However, there was no significant result for gene *pta* (PA0835), encoding the phosphate acetyltransferase responsible for the conversion of acety-CoA to acetyl-phosphate, which is the one missing link in whole pyruvate fermentation system. However, when assessing the raw microarray data the signal intensity is similar to PA0836, therefore it can be assumed that the whole pyruvate fermentation pathway is induced during tumour infection. Additionally, the fermentation of arginine through the arginine deaminase pathway was induced. The *arcDABC* genes have been recently shown to be co-regulated with genes responsible for pyruvate fermentation (Schreiber *et al.*, 2006). In the same report it is shown that there are two universal stress proteins linked to anaerobic survival with pyruvate fermentation (PA3309 and PA4352). Both of these were upregulated in the mouse tumour infection. Together with the overexpression of the pathways involved in anaerobic respiration there was inhibition of gene PA5435, encoding pyruvate carboxylase subunit B which is responsible for transformation of pyruvate to oxaloacetate and PA1580, encoding type II citrate synthase which transforms oxaloacetate to citrate, using acetyl-CoA. This clearly shows the direction of metabolism into pyruvate fermentation with the transformation, in the last instance, of acetyl-CoA to acetate recovering the ATP. Among the downregulated genes there were those involved in oxidative phosphorylation.

Other factors

Compared to the burn wound infection, ribosomal proteins PA3600 and PA3601 were highly overexpressed in a similar manner, but here there was no induction of the *np*20 gene or the genes encoding the zinc transporter system. Assessing the iron starvation response, there was overexpression of *hasAp* by 14-fold but not of the either siderophore systems. Another

similarity to the burn wound infection and the CF patient infection was the upregulation of the *betB* gene encoding betaine aldehyde dehydrogenase and the *betI* gene encoding a transcriptional regulator.

4.3.2.4.2. Testing mutant strains

To evaluate the use of the tumour infection as a model for *P. aeruginosa* infection, the following mutants were tested: (i) PA14 Δ*anr*, with the mutation in the global anaerobic regulator; (ii) PA14 Δ*cupA1*, to check if bacteria form biofilms similar to those isolates from a CF patient infection; and (iii) PA14 Δ*pqsA*, to analyse if PQS is necessary for the tumour infection. The analysis was based on the CFU obtained from a group of five mice per each strain and at each time point of infection. Figure 4-8 summarizes the survival of *P. aeruginosa* PA14 mutants and the wildtype strain in the tumour. At 24 hours p. i. there was no significant difference, but at 48 hours p. i. it was clear that the strain deficient in anaerobic regulation was unable to survive in the tumour. The strains with the mutation in the *pqsA* and *cupA1* genes were also in reduced numbers of more than 2-fold in comparison to the wildtype.

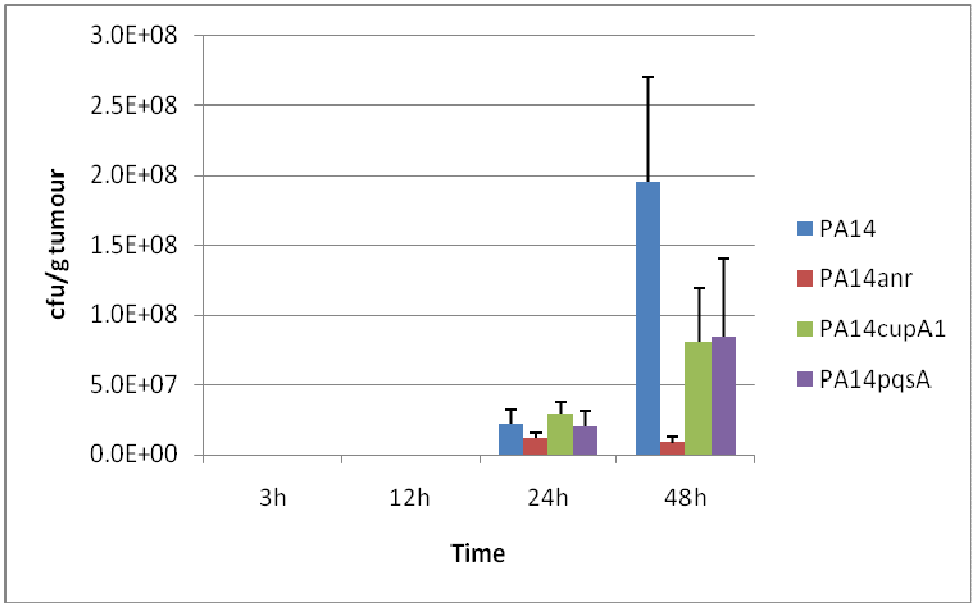


Figure 4-8: CFU of *P. aeruginosa* PA14 and mutants in the tumours of mice.

4.4. Comparing gene expression patterns across *in vivo* and *in vitro* settings

Up until this point the analysis of the microarray data has focused on assessing individual genes that were differentially regulated. In this chapter, the transcriptomic data was analysed by an unsupervised approach in order to find any internal structure or relationships over the global data set. Two approaches were taken: (i) agglomerative hierarchical clustering, which is a widely used method to consider clusters in data, and (ii) ordination via non-metric multidimensional scaling (nMDS), which assesses the closeness between conditions based on similarity in global transcriptomic data. As for principal component analysis (PCA) non-metric MDS illustrates the similarity or difference across conditions in both magnitude and direction to each other. The gene expression from five conditions the burn wound infection, the tumour infection model, the lettuce infection model, planktonic growth and biofilm growth were compared.

4.4.1. Hierarchical clustering

Agglomerative hierarchical clustering was first used to assess the similarities in gene expression among duplicate independent clones at each condition. The five conditions are presented in Figure 4-9. This dendogram shows that duplicates from each of the three independent clones within each condition indeed cluster together. This indicates that the gene expression of each independent clone at each condition is more similar to the other two clones at the same condition rather than the same clone at under a different infection conditions. For example, the gene expression of clone PBCLOp11 always clusters with clones PBCLOp17 and PBCLOp10 irrespective of the infection condition. Although the same clones were used across the conditions, their gene expression is dependent upon the condition and not the clone itself. Interestingly, under the conditions of the lettuce infection model, the planktonic growth and the tumor infection model, clones PBCLOp17 and PBCLOp10 were always more similar than clone PBCLOp11. While under the conditions of the burn wound infection and biofilm growth, clones PBCLOp10 and PBCLOp11 were always more similar

than clone PBCLOp17. This is important as future analysis rely upon these three clones forming the predefined groups of each condition. This also shows that the gene expression profiling itself produces consistent and reliable data.

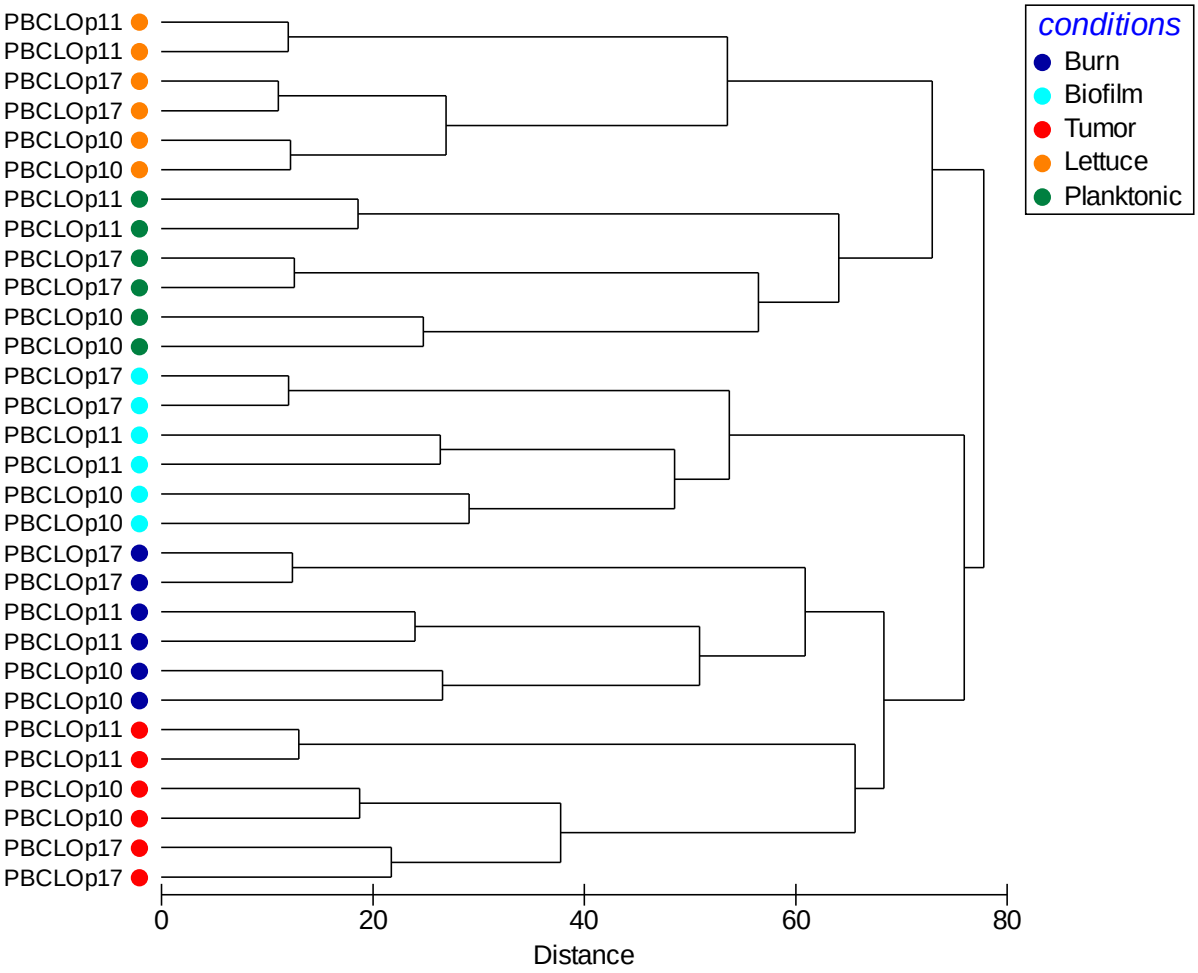


Figure 4-9: Hierarchical clustering of the conditions.

To extend upon the visualisation of the data, cluster analysis was also performed on the genes. Figure 4-10 shows the clustering of the genes connected with a heat map. Visually, it can be seen that there are specific clusters of genes unique for each conditions.

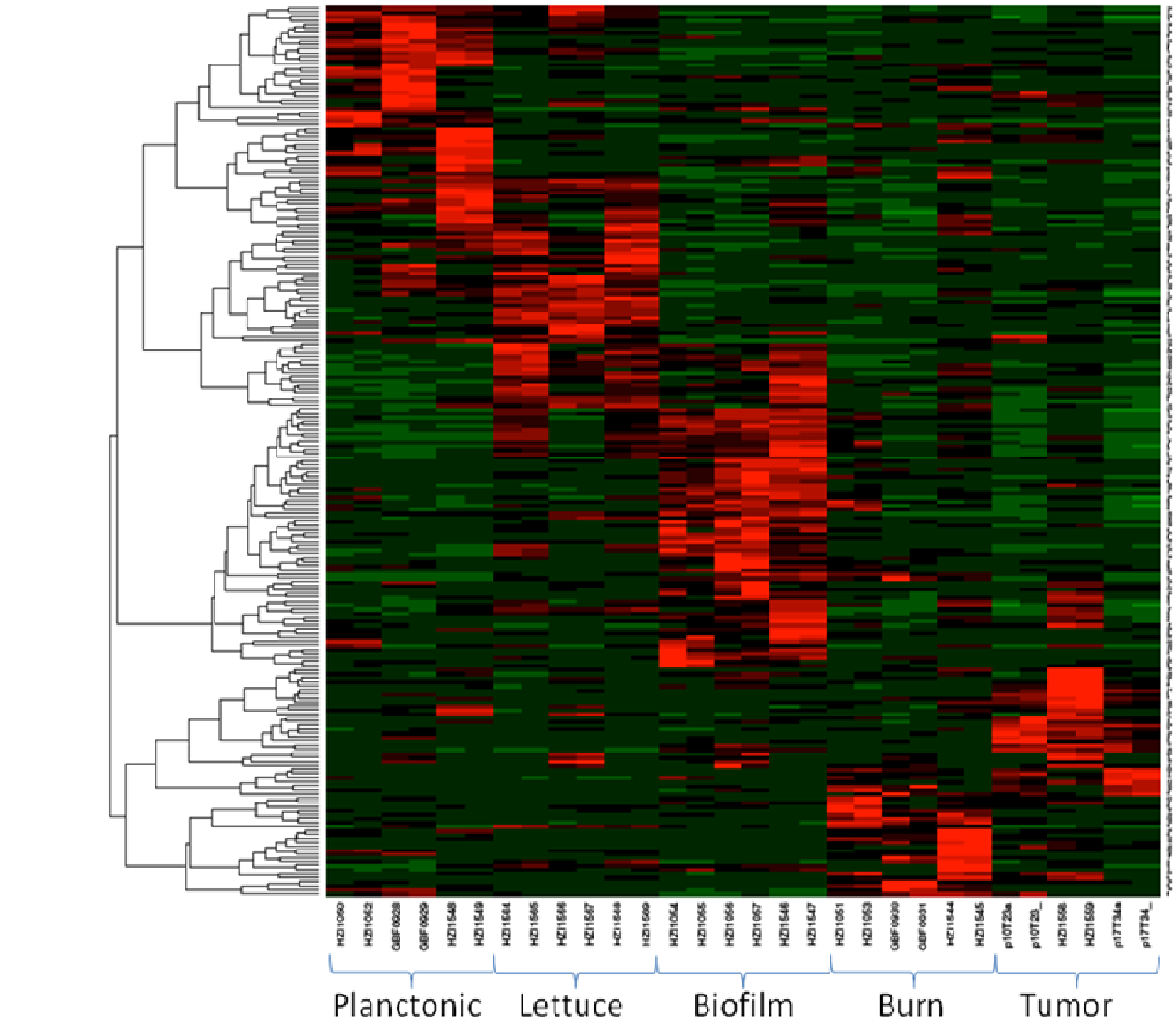


Figure 4-10: Hierarchical clustering of genes presented with the heat map of expression values.

4.4.2. Finding patterns across different infection conditions using a global analysis approach

Multidimensional scaling (MDS) is an ordination routine used to observe actual distances, patterns, degrees of similarity and dissimilarity between samples. That is, the distance in magnitude and direction between each condition on a MDS plot is equivalent to its difference and change in global gene expression respectively. Figure 4-11 shows how the five conditions relate to each other based on their similarity of gene expression (global transcriptomic pattern). After calculation of the Euclidean distance between each pair of samples (including duplicates of each clone and replicates of each condition), non parametric multi-dimensional scaling plots (nMDS) were generated and ANOSIM was used to test for statistically significant differences between each of the conditions. The stress value of the MDS plot is 0.15 and indicates a good ordination.

Firstly, as observed in agglomerative hierarchical clustering, the duplicates of each clone within each condition group together. Secondly, clones from the tumour infection and clones from the burn wound infection ordinate together at the centre top of the MDS (red and dark blue dots representing tumour infection model and burn wound infection respectively), while clones from the planktonic growth (green) ordinate to the left of the plot, biofilm (light blue) ordinate to the lower right of the plot and lettuce infection model (brown) ordinate together at the bottom of the plot. This is confirmed with a one-way ANOSIM, where all conditions are significantly different to each other ($p < 0.05$). Furthermore, the R-statistic value of the pairwise comparison between the tumor infection and burn wound infection is 0.752 and is lower than all the of the other pairwise comparison indicating that these two conditions are more related to each other based on their global gene expression profiles (Table 4-7).

The growth conditions can be regarded as clearly different, although there was some overlapping between burn wound infection and tumour infection clones. In this work the biofilm and planktonic growth conditions are used as controls. The infection conditions are

lettuce, tumour and burn wound. The lettuce infection was much more different than the burn wound and the tumour infection with R-statistics of 1 and 0.941 respectively.

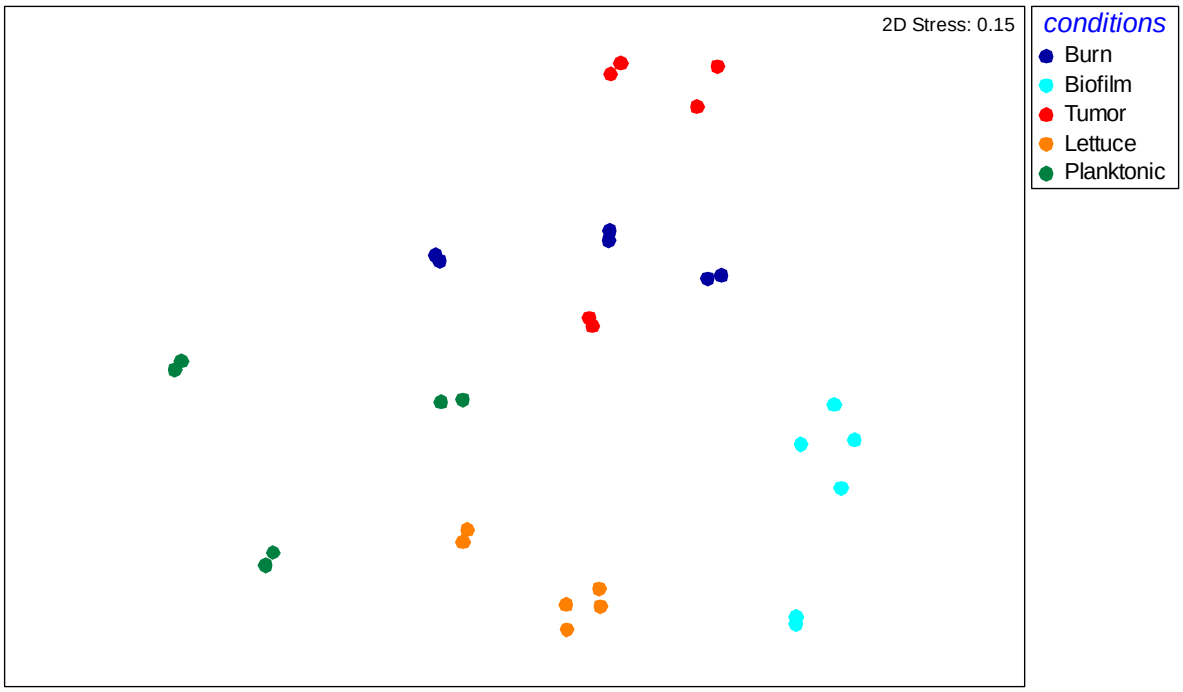


Figure 4-11: Multidimensional scaling (nMDS) plot based on Euclidean distance of gene from different conditions. A stress value of 0.15 indicates good ordination.

Table 4-7: The one-way ANOSIM pairwise comparisons of *P. aeruginosa* growth conditions.

Pairwise comparison	R statistic	p - value
Burn/Tumour	0.752	0.002
Planktonic/Lettuce	0.881	0.002
Planktonic/Burn	0.911	0.002
Biofilm/Tumour	0.941	0.002
Tumour/Lettuce	0.941	0.002
Planktonic/Tumour	0.974	0.002
Planktonic/Biofilm	0.985	0.002
Burn/Biofilm	0.996	0.002
Biofilm/Lettuce	1	0.002
Burn/Lettuce	1	0.002

Thus, it is interesting that the two more realistic infection ordinate closer together while the other conditions are more distinctly separated. SIMPER analysis was used to assess those genes that contribute to the greatest differences between tumour infection and burn wound infection. Firstly, SIMPER was used to indicate which genes accounted the most for the difference between the tumour and burn wound conditions. Table 4-8 shows those genes that contribute more than 0.40 % (of 1677 genes analysed) to the dissimilarity between these conditions. The gene that shows the greatest difference was the *bfd* gene encoding for bacterioferritin-associated ferredoxin. The gene *bfd* was more highly expressed in the burn wound infection than the tumour infection (11.50 compared to 6.17, respectively). This confirms a strong iron starvation response present during the burn wound infection. The same gene has been found to be overexpressed by 200-fold in an iron starvation experiment of Ochsner and colleagues (2002). Another gene that had a higher expression level in the burn wound and is also involved in iron starvation was *pvdS*. Furthermore, the gene PA4570 is also induced by iron. Other genes listed are PA3600, which encodes an alternative ribosomal protein, the gene PA4063 from the cluster of putative secretom system induced during burn wound infection. However, most of the genes encoded hypothetical and conserved hypothetical proteins.

Table 4-8: Similarity Percentage (SIMPER) analysis assessing those genes that account for the greatest dissimilarity between burn wound infection and tumour infection model. Genes having a contribution of more than 0.40 % are presented.

Locus ID	Gene	Product Name	Burn Expression value	Tumour Expression value	Contribution %	Cumulative %
PA3530	*bfd*	bacterioferritin-associated ferredoxin	11.50	6.17	0.64	0.64
PA3572		hypothetical protein	4.09	9.14	0.61	1.25
PA2146	*yciG*	conserved hypothetical protein	12.00	7.48	0.59	1.84
PA3600	*rpl36*	conserved hypothetical protein	13.30	8.66	0.53	2.37
PA2159		conserved hypothetical protein	10.40	5.97	0.51	2.87
M57501	*flaA*	flagellin type A	7.70	5.27	0.49	3.37
PA0713		hypothetical protein	5.23	9.95	0.49	3.85
PA1852		hypothetical protein	9.37	5.42	0.47	4.80
PA4063		hypothetical protein	7.02	2.40	0.46	5.26
PA4570		hypothetical protein	11.40	6.94	0.46	5.72
PA4141		hypothetical protein	9.77	8.91	0.45	6.17
PA2426	*pvdS*	sigma factor PvdS	8.86	4.53	0.41	6.58
PA3719		hypothetical protein	6.54	4.45	0.41	6.99

4.4.3. Comparing the CF pulmonary infection to the other infection conditions

This section takes a preliminary look at how different the CF patient infection is compared to the other infection conditions. This work is considered preliminary because only one isolate from one CF patient was analysed here, while all other conditions sort three independent isolates. The global gene expression of this one isolate was ordinated with the gene expression profiles of all of the *in vivo* and *in vitro* conditions. Figure 4-12 presents the new nMDS plot showing how the CF pulmonary infection compares to other conditions. The CF patient infection isolate is more closely related to the biofilm, burn and tumour infection settings than to the lettuce or planktonic growth. Also, it could be argued that there is a

gradient from the left to the right side of the plot, from planktonic to CF. Perhaps it can be hypothesised that this gradient represents the increase in virulence of *P. aeruginosa* across different growth conditions. If this is the case, then the biofilm growth condition is remarkable and strengthens the view that the ability for biofilm formation *per se* is a virulence factor responsible for successful infection.

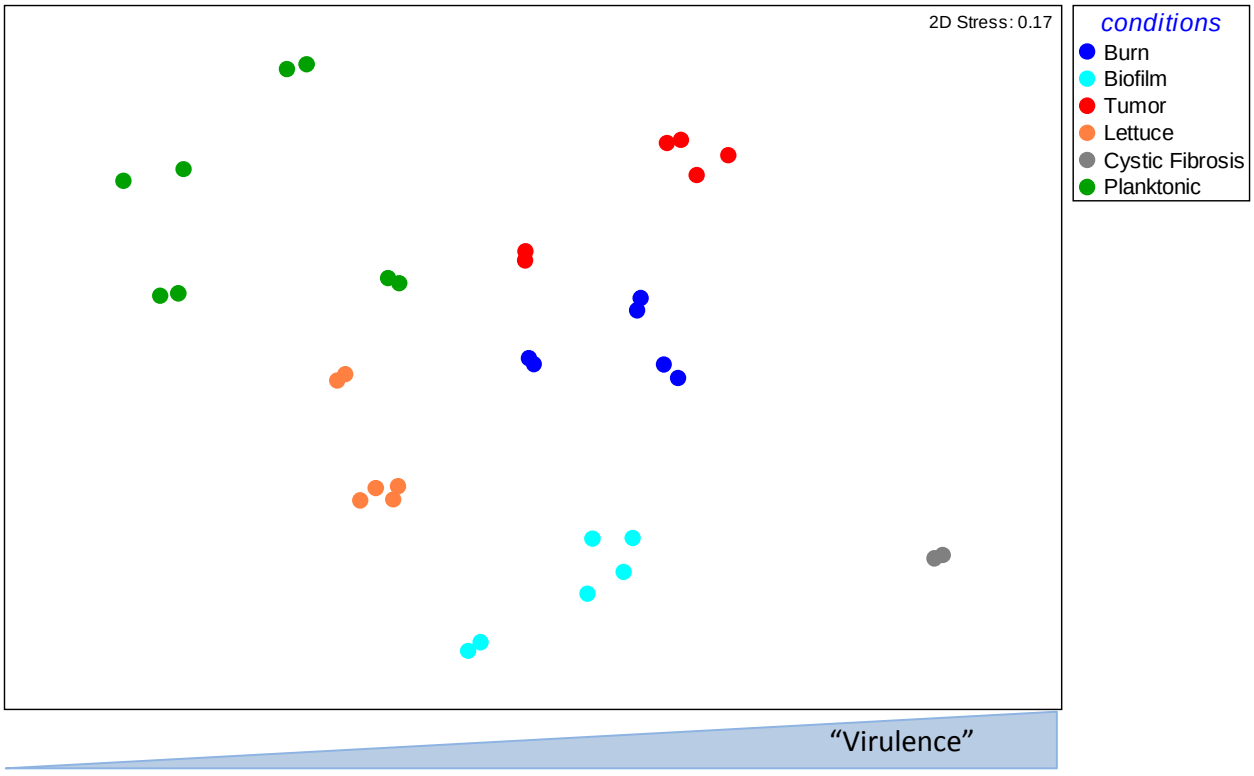


Figure 4-12: Multidimensional scaling (nMDS) plot based on Euclidean distance of 1677 genes from different conditions. The stress value is 0.17 , which indicates a good ordination.

4.4.4. Overlaying the expression values of a single gene over the global pattern

The relationship between the pattern in an MDS and a univariate variable (the gene expression value) can be visualised using “bubble plots”. This is achieved by presenting the value of the univariate variable as a symbol of different sizes and superimposing it onto the ordination of its corresponding sample. The genes previously discussed in the chapter 4.3 were analysed with the MDS bubble plots. Figure 4-13 presents the 8 MDS plots which are based on the MDS from Figure 4-12, with the expression values from 8 given genes at each infection condition. The 8 genes are: *rhlR*, *lasR*, *pqsA*, *hasAp*, PA4835, PA3600, *exoT* and *cupA1*, and are representatives of an array of different processes within the cell, such as: QS, iron acquisition, zinc homeostasis, type III secretion system and biofilm formation potential, respectively. For example the genes *cupA1* and *exoT* were visually most overexpressed in the mouse tumour infection model (Figure 4-13a and d), whereas *las* and *rhl* genes of the QS system seem to have no overall (Figure 4-13b and c). However, the *pqsA* gene (Figure 4-13h) is significantly downregulated in the CF patient infection. The genes *hasAp*, PA3600 and PA4835 are clearly overexpressed in mammalian infection conditions only, that is the burn wound, CF patient and mouse tumour infection but not in the lettuce infection model (Figure 4-13e, f and g). Except for the *pqsA* gene (Figure 4-13h), most values of these selected genes are equivalent between the CF and the burn wound infection.

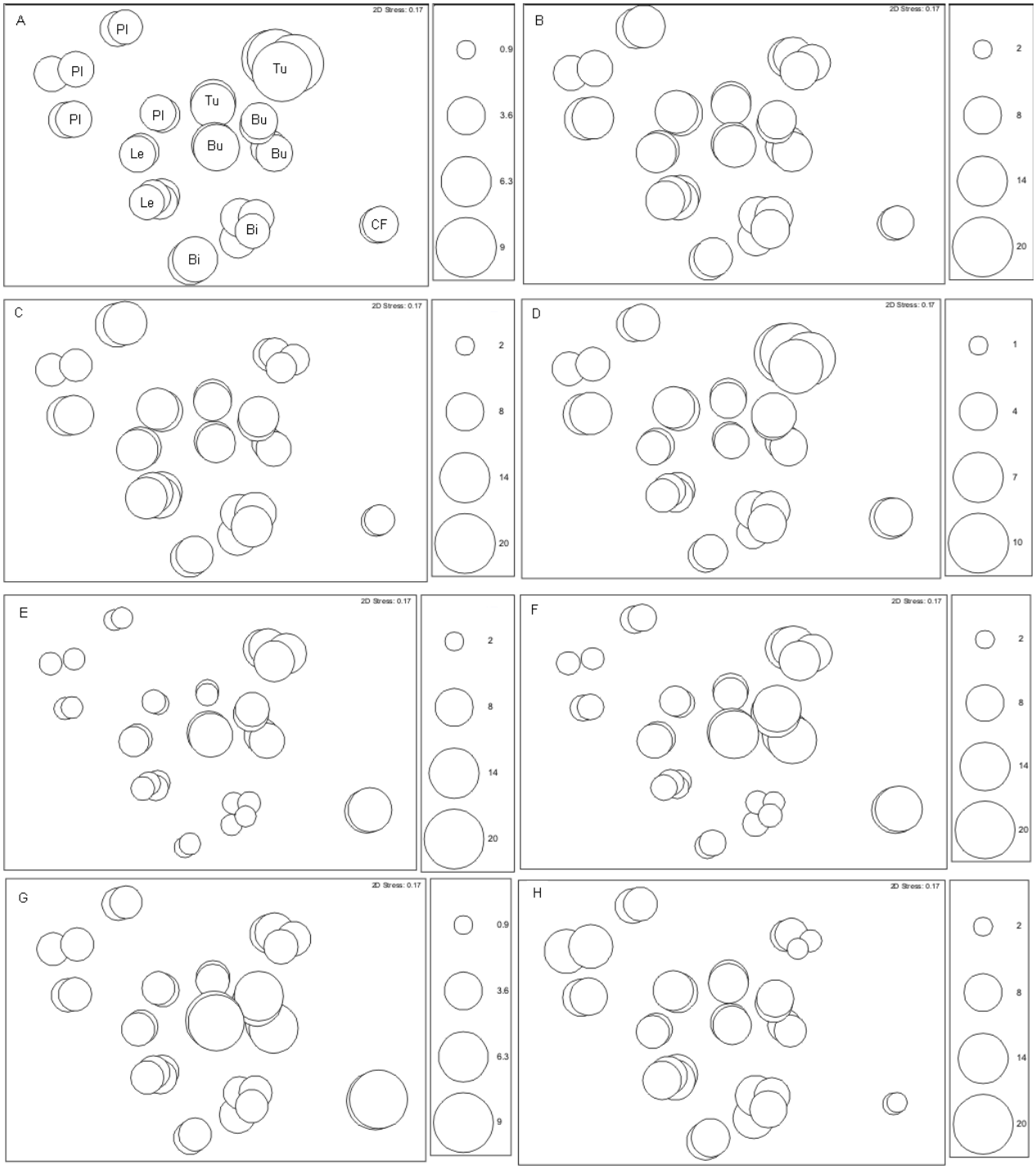


Figure 4-13: 2D nMDS bubble plots showing all of the infection conditions and bubbles corresponding to the expression value of each gene: A) *cupA1*, B) *lasR,* C) *rhlR*, D) *exoT*, E) *hasAp*, F) PA3600, F) PA4835 and F) *pqsA.* On the first bubble plot the conditions are indicated and apply to all plots: Pl – Planktonic, Bi – Biofilm, Le – lettuce infection, Tu – mouse tumour infection, Bu – burn wound infection, CF – CF paitent infection

In summary, using these individual genes as indicators of the different processes within the *P. aeruginosa* cells, it can be confirmed that:

- The QS systems *las* and *rhl* are generally unchanged across all conditions
- The heme uptake is greatest in the burn wound infection, the CF patient infection and the mouse tumour infection
- The gene encoding ribosomal protein, which expression is related to zinc homeostasis is overexpressed across the mammalian infections (burn wound, CF patient and mouse tumour infection)
- Type III secretion system and gene cupA1 (involved in biofilm formation during infection) are most induced in the mouse tumour infection model

Such unsupervised analysis approaches provide a valuable tool to visually and statistically evaluate the infection conditions, to find global patterns as well as similarities and dissimilarities across a broad range of *in vivo* and *in vitro* conditions. Then, information from a supervised approach (i.e. interesting individual genes), being the value of the individual gene can be superimposed with the unsupervised approach to further determine any specific patterns in the data. It is aimed that such an approach can better evaluate: (i) the comparison of infection models to the real infections, and (ii) similarities between the *in vivo* infections, to better understand global pathogen behaviour upon infection.

4.5. The quest for anti-pseudomonal compounds

The number of multi-drug resistant *Pseudomonas* strains is constantly increasing, making the treatment of infections of those very difficult as antibiotic treatment may not be sufficient in such cases. Therefore, it is important to find novel prevention strategies that may act on bacterial cells by directly targeting the virulence of pathogens rather than (or in addition to) their growth. This approach helps to overcome the main problem occurring in antibiotic treatment, which is selectivity of resistant mutants that leads to selection of multiresistant clones (Hentzer *et al.,* 2003). In case of *P. aeruginosa* a natural target is the QS system. There are extensive studies assessing natural and synthetic compounds with QS inhibitory activity (Rasmussen *et al.,* 2005a, Bjarnsholt *et al.,* 2005).

Protoanemonin (4-methylenebut-2-en-4-olide) is one of the "dead end" products of the degradation of polychlorinated biphenyls (Bruckmann *et al.*, 1998). It is formed by misrouting of chlorocatechol into the ordinary catechol pathway where muconate cycloisomerase forms protoanemonin from 3-chloromuconate as substrate. Protoanemonin is also a natural product from plants of the *Ranunculaceae* family and it possesses an antibiotic activity (Blasco *et al.*, 1995).

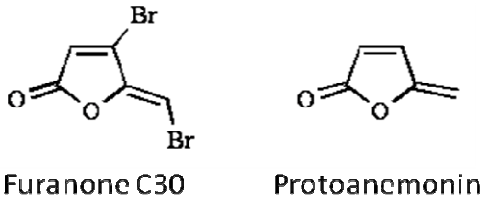


Figure 4-14: Comparing the chemical structures of furanone C30 and protoanemonin.

Protoanemonin also has similar structure to furanone C30, which is known to inhibit QS (Hentzer *et al.,* 2003). Figure 4-14 presents structures of both compounds. Protoanemonin was synthesised (Bobadilla, 2006) with the technique established by Crey and colleagues (2004) and analysed using the QS inhibitory screening system developed by Hentzer *et al*.

(2002). It uses the QS monitor strain *P. aeruginosa* MH602 consisting of a reporter gene (*gfp* (ASV)) fused to the promoter -including the *lux* box homologue - of *lasB* being controlled by quorum sensing in *P. aeruginosa*. The half-life of Gfp (ASV) is approximately 110 minutes allowing online monitoring of changes in gene expression to be seen over a time span of a few hours.

When the gene encoding the modified Gfp (ASV) is fused to a promoter positively regulated by quorum sensing (such as the *lasB* promoter), elevated expression of the quorum sensing controlled gene can be measured as an increase in fluorescence. The addition of a quorum sensing inhibitor, to this construction will result in a lowered expression of Gfp (ASV) to an extent proportional to the efficiency of the inhibitor (Hentzer *et al.,* 2002). Work performed by colleagues at DTU Biocentrum showed that this compound strongly inhibited QS at concentrations of protoanemonin in the range of 40μM to 174μM without impact on the growth (Figure 8-1 in the appendix).

4.5.1. Transcriptional profiling of PAO1 in response to protoanemonin

A QS-inhibition screening assay suggested that QS circuits are targeted by protoanemonin at a concentration range not inhibitory to bacterial growth. In order to investigate if this compound can target other factors in *P. aeruginosa* two approaches were followed: i) transcription profiling of *P. aeruginosa* PAO1 in response to protoanemonin and ii) proteomic analysis of secreted proteins in response to protoanemonin. The experiments were made using *P. aeruginosa* PAO1 for easy comparison to already published reports on QS inhibitors.

During treatment with protoanemonin 84 genes were differentially expressed, whereby 46 of these were induced and 38 were repressed by the action of protoanemonin. Table 4-9 represents those genes that were significantly downregulated in response to protoanemonin. The majority of these repressed genes have been previously shown to be regulated by the *las* or *rhl* system (Schuster *et al.,* 2003) or known to be regulated during different growth phases (Wagner *et al.,* 2003). Genes *lasA* and *lasB* encoding LasA protease

precursor and elastase LasB are major effectors of the *las* QS system. Following the QS regulated pathways, the phenazine biosynthesis is also repressed in this current work. A total of 29 represed genes (76.3% of 38) have been previously reported to be QS regulated at the transcriptomic level and 13 (34.2% of 38) have been also repressed by furanone C30. Additionally, a gene encoding for catalase has been shown to be controlled by QS in response to hydrogen peroxide (Hassett *et al.*, 1997).

Surprisingly, the gene *exoS* encoding exoenzyme S secreted via the type III secretion system was dowregulated in response to protoanemonin. Previously, T3SS was reported to be negatively regulated by *rhl* system, where mutants of *rhlR* have showed upregulation of the T3SS (Hogardt *et al.*, 2004, Bleves *et al.*, 2005).

Table 4-9: Genes downregulated in response to protoanemonin. Comparison with previously reported QS regulated genes: * indicates those QS regulated genes in wild type versus *lasR*, *rhlR* mutant (Schuster *et al.*, 2003), # indicates those QS regulated genes in early stationary phase and ^ indicates mid logarithmic phase (Wagner *et al.*, 2003), – indicates those genes repressed by furanone C30 (Hentzer *et al.*, 2003).

PA Number	Gene	Fold change	Product Name	QS	C30
PA0795	*prpC*	-9.44	citrate synthase 2		
PA0852	*cbpD*	-13.04	chitin-binding protein CbpD precursor	*	-
PA0865	*hpd*	-5.24	4-hydroxyphenylpyruvate dioxygenase		
PA1174	*napA*	-6.23	periplasmic nitrate reductase protein NapA		
PA1871	*lasA*	-16.81	LasA protease precursor	*#	-
PA1901	*phzC2*	-15.53	phenazine biosynthesis protein PhzC	#	-
PA1902	*phzD2*	-16.86	phenazine biosynthesis protein PhzD	#	-
PA1903	*phzE2*	-22.94	phenazine biosynthesis protein PhzE	#^	-
PA1904	*phzF2*	-24.57	probable phenazine biosynthesis protein	#^	-
PA1905	*phzG2*	-20.58	probable pyridoxamine 5'-phosphate oxidase	#^	
PA1984	*exaC1*	-4.89	probable aldehyde dehydrogenase	#	
PA1999	*pcaI*	-11.30	probable CoA transferase, subunit A	^	
PA2007	*maiA*	-13.48	maleylacetoacetate isomerase	*	
PA2008	*fahA*	-8.95	fumarylacetoacetase	*	
PA2009	*hmgA*	-6.38	homogentisate 1,2-dioxygenase	*	
PA2067		-5.28	probable hydrolase	*	
PA2068		-5.40	probable major facilitator superfamily (MFS) transporter	*#	-
PA2069		-7.92	probable carbamoyl transferase	*#	-
PA2302		-6.26	probable non-ribosomal peptide synthetase	*#	-

PA2565		-4.89	hypothetical protein	*#	
PA3183	*zwf*	-4.90	glucose-6-phosphate 1-dehydrogenase	*	
PA3195	*gapA*	-5.33	glyceraldehyde 3-phosphate dehydrogenase	*	
PA3724	*lasB*	-26.39	elastase LasB	*#^	-
PA3841	*exoS*	-5.93	exoenzyme S		
PA4078		-8.67	probable nonribosomal peptide synthetase	*	
PA4206	*mexH*	-6.77	probable Resistance-Nodulation-Cell Division (RND) efflux membrane fusion protein precursor	*	
PA4209	*phzM*	-11.81	probable phenazine-specific methyltransferase	*#	-
PA4210	*phzA1*	-19.12	probable phenazine biosynthesis protein	*	
PA4211	*phzB1*	-24.57	probable phenazine biosynthesis protein	*#^	
PA4217	*phzS*	-35.59	flavin-containing monooxygenase	*#	-
PA4236	*katA*	-5.07	catalase		
PA4366	*sodB*	-6.17	superoxide dismutase		
PA4587	*ccpR*	-4.81	cytochrome c551 peroxidase precursor	#	
PA4810	*fdnI*	-5.18	nitrate-inducible formate dehydrogenase, gamma subunit		
PA4811	*fdnH*	-10.32	nitrate-inducible formate dehydrogenase, beta subunit		
PA5172	*arcB*	-5.16	ornithine carbamoyltransferase, catabolic		
PA5173	*arcC*	-8.87	carbamate kinase		
PA5220		-5.14	hypothetical protein	*#^	-

While the response of the *P. aeruginosa* to furanone C-30 induces only 8 genes from a total of 93 differentially regulated genes (Hentzer *et al.,* 2003), protoanemonin induced more than the 50% of all differentially regulated genes in the analysis. Interestingly, 68% of these genes are related to iron starvation (Table 4-10) previously reported by Ochsner *et al.,* (2002). These genes include pyoverdine and pyochelin synthesis pathways involving genes *pvd* and *pch* respectively. Also, genes *fumC-sodM* encoding fumarase C and manganese-cofactored superoxide dismutase which are controlled by the *fur* operon and are elevated in mucoid, alginate-producing bacteria (Hassett *et al.*, 1997).

Table 4-10: Genes upregulated in response to protoanemonin. * indicates those genes induced in iron starvation response by Ochsner *et al.,* (2002).

PA Number	Gene	Fold change	Product Name	Iron
PA0201		12.35	hypothetical protein	

PA0284		7.05	hypothetical protein	
PA0472	*fiuI*	6.97	probable sigma-70 factor, ECF subfamily	*
PA0672	*hemO*	13.15	heme oxygenase	*
PA0802		18.46	hypothetical protein	
PA1300		13.18	probable sigma-70 factor, ECF subfamily	*
PA2385	*pvdQ*	8.31	PvdQ	*
PA2386	*pvdA*	7.10	L-ornithine N5-oxygenase	*
PA2391	*opmQ*	9.31	probable outer membrane protein precursor	*
PA2392	*pvdP*	11.62	PvdP	*
PA2394	*pvdN*	8.48	PvdN	*
PA2395	*pvdO*	8.84	PvdO	*
PA2397	*pvdE*	7.54	pyoverdine biosynthesis protein PvdE	*
PA2399	*pvdD*	6.98	pyoverdine synthetase D	*
PA2402		9.35	probable non-ribosomal peptide synthetase	*
PA2412		7.46	conserved hypothetical protein	*
PA2413	*pvdH*	7.80	L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase	*
PA2424	*pvdL*	12.09	PvdL	*
PA2425	*pvdG*	10.27	PvdG	*
PA2427		15.95	hypothetical protein	*
PA2486		10.16	hypothetical protein	
PA2663		10.01	hypothetical protein	
PA2664	*fhp*	34.31	flavohemoprotein	
PA2759		14.63	hypothetical protein	
PA3126	*ibpA*	9.65	heat-shock protein IbpA	
PA3446	*ssuE;*	9.69	conserved hypothetical protein	
PA3815		13.87	conserved hypothetical protein	
PA3899		8.13	probable sigma-70 factor, ECF subfamily	*
PA4218		10.54	probable transporter	*
PA4219	*yfpB*	17.91	hypothetical protein	*
PA4220	*fptB*	8.41	hypothetical protein	*
PA4224	*pchG*	8.76	pyochelin biosynthetic protein PchG	*
PA4225	*pchF*	6.97	pyochelin synthetase	*
PA4226	*pchE*	7.81	dihydroaeruginoic acid synthetase	*
PA4229	*pchC*	7.99	pyochelin biosynthetic protein PchC	*
PA4387	*fxsA;*	7.34	conserved hypothetical protein	
PA4467		18.96	hypothetical protein	*
PA4468	*sodM*	16.32	superoxide dismutase	*
PA4469		8.27	hypothetical protein	*
PA4470	*fumC1*	12.12	fumarate hydratase	*
PA4471	*fagA*	7.35	hypothetical protein	*
PA4570		18.65	hypothetical protein	*
PA4610		7.69	hypothetical protein	

PA4623	10.62	hypothetical protein
PA4881	7.14	hypothetical protein

4.5.2. Proteomic analysis of secreted proteins in response to protoanemonin

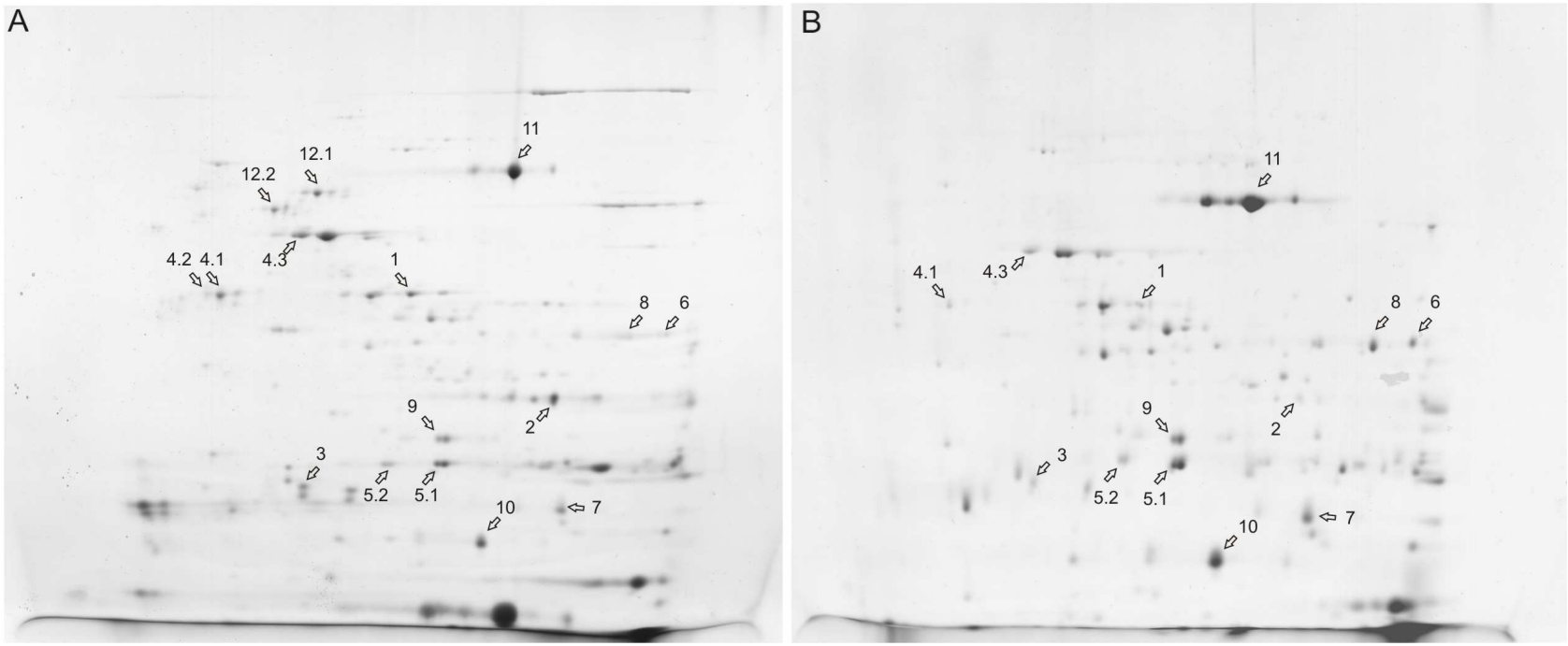
In order to further elucidate the results obtained from the transcriptomic approach, proteomic analysis was also performed on the secreted proteins fraction of *P. aeruginosa* in response to protoanemonin. *P. aeruginosa* PAO1 was grown in ABT medium with casamino acids and the experiment performed with 125 μM of protoanemonin. Extracellular proteins from all replicates of *P. aeruginosa* cultures were solubilised and separated using two-dimensional gel electrophoresis (2-DE) across a pH range 4-7 (Figure 4-15). Image and statistical analyses, using the Z3 software packages were performed to detect differences in protein expression between bacterial cells treated and untreated with protoanemonin. The list of identified proteins is given in Table 4-11.

In comparison to the transcritpomic profiles there was consistency between two main groups of regulated genes in the *las* system and iron starvation response. Firstly, the elastase LasB protein was downregulated in protoanemonin treated samples while the PvdO protein (pyoverdine) was upregulated. Following are those proteins which were downregulated: (i) the probable bacteriophage protein (spot 1); (ii) the flagellin type B (spots 4.1, 4.2); (iii) additionally there are two proteins present only in the control, flagellin type B (spot 4.3) and probable aminopeptidase encoded by PA2939 (spots 12.1, 12.2). Among the upregulated genes were: (i) arginine/ornithine binding protein (spots 5.1, 5.2); (ii) sulfate binding protein of ABC transporter (spot 6); (iii) thiol:disulfide interchange protein DsbA (spot 7); (iv) the polyamine transport protein (spot 8); (v) the hypothetical protein encoded by PA0423 and (vi) a cation transport ATPase, which was identified with the best score from bacterium *Listeria welshimeri* serovar 6b str. SLCC5334

Table 4-11: Identification of extracellular proteins form PAO1 differentially expressed in response to protoanemonin. Proteins were identified by peptide mass mapping. Spot numbers refers to those shown in Fig. 4-15. # indicates those proteins present only on the control 2D gel, * indicates the identified as proteins from *Listeria welshimeri* serovar 6b str. SLCC5334

Spot no.	PA no.	Gene	Protein identification	Matching peptides	Sequence coverage %	Total pI	Total mass (kDa)	Differential expression in protoanemonin
1	PA0622		Probable bacteriophage protein	8	28	5.80	20.66	-7.19
2	PA3724	*lasB*	Elastase	15	48	5.79	33.12	-5.85
3	PA1094	*fliD*	Flagellar capping protein FliD	10	35	6.52	43.42	-5.13
4.1	PA1092	*fliC*	Flagellin type B	21	38	5.40	49.22	-4.65
4.2	PA1092	*fliC*	Flagellin type B	17	36	5.40	49.22	-2.42
4.3	PA1092	*fliC*	Flagellin type B	15	27	5.40	49.22	#
12.1	PA2939		Probable aminopeptidase	13	25	5.02	57.48	#
12.2	PA2939		Probable aminopeptidase	13	22	5.02	57.48	#
5.1	PA0888	*aotJ*	Arginin/ornithine binding protein	11	54	6.43	27.99	2.40
5.2	PA0888	*aotJ*	Arginin/ornithine binding protein			6.43	27.99	4.61
6	PA1493	*cysP*	Sulfate-binding protein of ABC transporter	13	32	7.77	36.50	2.64
7	PA5489	*dsbA*	Thiol:disulfide interchange protein DsbA	11	46	5.98	23.36	2.76
8	PA0300	*spuD*	Polyamine transport protein	11	29	7.00	40.71	2.85
9	PA2395	*pvdO*	PvdO	10	38	5.41	31.05	5.32
10	PA0423		Hypothetical protein	8	35	6.01	20.76	10.88
11			Cation transport ATPase*	12	18	5.02	95.80	17.81

Figure 4-15: Extracellular proteins from *P. aeruginosa* PAO1. (A) control conditions, (B) protoanemonin at 125 µM. Proteins were separated by 2-DE using IPG ranges of pH 4-7. Proteins discussed in the text are highlighted and were identified by peptide mass mapping. Numbers represent the spot identifications listed in Table 4-11.



5. Discussion

Pseudomonas aeruginosa is a threatening opportunistic pathogen and one of the major agents of nosocomial infections in immunosupressed patients. It is also a common environmental strain isolated from soil and wet niches. There are relatively small differences between strains cultivated from environmental and clinical samples and both of them may be similarly virulent (Alonso *et al.*, 1999). In this work, a global transcription profiling approach was used to identify the factors that may be responsible for a switch from an environmental life style to a pathogenic state, in which *P. aeruginosa* cells have high impact on mortality of infected patients.

Ideally such analyses are to be performed on *in vivo* samples taken from patients. However, it is usually very difficult to obtain from these samples enough RNA for global expression profiling. Furthermore, the amount of sample itself is often limited. By adapting and newly developing protocols and methods for sample collection, transportation, enrichment and microarray preparation in this work, we succeeded in profiling *in vivo* gene expression of *P. aeruginosa* infecting burn wounds, CF patients and different infection models.

Having appropriate models that mimic closely the real infection are crucial to perform experiments for testing hypotheses on infection mechanisms or on the effect of potential novel drug targets. Two infection models were tested in this work: a recently reported lettuce infection model and a tumour mouse infection model specifically developed here.

Planktonic and biofilm cultures grown in rich medium were used as control experiments. Planktonic cells were harvested in the early stationary phase, while biofilms were collected after 24 h of growth. These control conditions were chosen to mimic a nutrient richness similar to the infection sites, which is abundant in amino acids. The analyses provided the chance to pin-point differentially regulated genes involved in infection. The major changes observed in the analysis of the transcriptomic data were those involved in acquisition of trace elements such as iron and zinc, genes coding for proteins involved in alginate

production, anaerobic growth, type III secretion system and number of hypothetical operons not previously known to be connected with virulence.

5.1. Minerals and trace elements

The efficient infection of a host by bacteria requires that they develop appropriate survival strategies to overcome or bypass immune defences and to acquire essential nutrients such as iron and zinc. Iron acquisition is illustrative of how bacteria overcome such limitations during host infection. Iron availability is strictly controlled in eukaryotes by metal binding proteins (e.g. ferritin, transferritin and lactoferin), which prevents its reactivity and limits the availability for uptake by pathogens (Payne, 1993, Ratledge & Dover, 2000, Schaible & Kaufmann, 2004). Since iron plays crucial catalytic roles in a large number of proteins in bacteria, they have developed sophisticated systems for iron acquisition (Poole & McKay, 2003), which have been extensively studied and well reported. We propose in the current study that acquisition of another trace element, zinc, is important for bacterial cells during burn wound infections.

Iron

The study of iron acquisition systems *in vitro* by transcription profiling has been done in the past by assessing the response of cells under two main conditions, namely i) iron starvation (Ochsner *et al.,* 2002) and ii) addition of iron to the medium with iron-starved cells (Palma *et al.,* 2003). In this work, differentially regulated genes in response to iron starvation were observed in all of the *in vivo* infection conditions, with the most complex pattern being that of the burn wound infection setting, where 50% of the genes previously shown to be regulated by iron starvation were induced during the burn wound infection. These include the genes encoding for ECF sigma factors: PvdS, FiuI, FemI, PA1300 and PA4896. The expression of these regulators and 46 other genes regulated by Fur regulator demonstrates that iron acquisition is crucial for survival of *P. aeruginosa* infecting a burn wound. The genes from PA4834-PA4837 were also consistently upregulated in both the burn wound infection and the CF patient infection. According to the Pseudomonas Genome Database and similarity searches, this operon putatively encodes for a probable novel siderophore system.

Thus, it may be a novel system involved in infection. A number of other reports in the literature support this hypothesis: i) this operon has been shown to be upregulated in *P. aeruginosa* PA14 growing on the artificial sputum medium as a carbon source (Palmer *et al.*, 2005); ii) the gene PA4837 has been reported to be expressed in *P. aeruginosa* clinical CF isolates in the early CF infection by phage display (Beckmann *et al.*, 2005); and iii) PA4837 was also reported to be upregulated in an *in vivo* peritoneal infection in rats (Mashburn *et al.,* 2005).

The ferric uptake regulator Fur was recently reported to have a broader role than previously expected. It has been shown to negatively regulate the small RNA (sRNA) PrrF1 and PrrF2, which are post-transcriptionally repressing a number of metabolic genes. As a result of the Fur activity, these genes are induced during iron starvation (Vasil, 2007). In the burn wound infection experiments, this was observed and will be discussed further in section 5.2.

Iron starvation response genes are also upregulated in *P. aeruginosa* infecting lettuce. The main iron starvation response sigma factor PvdS is overexpressed together with the ECF sigma factors PA0472, PA1912, PA2486 and.

In contrast to the other infection conditions tested, *P. aeruginosa* infecting mouse tumours did not exhibit strong iron starvation response and only a gene *hasAp* encoding for a heme acquisition protein was induced. This may be due to the presence of a necrotic compartment in the tumour, where degraded eukaryotic cells and debris may release sufficient amounts of iron so that there is no need for upregulation of the iron-starvation response genes. The heme acquisition protein encoding gene was upregulated in all mammalian infection settings and not in the plant model, as was expected and shows the careful regulation of such a complicated system as iron acquisition. Thus, in short, the global transcription analyses revealed the important role of iron acquisition among most of infection settings. The most severe iron limitation occurred in burn wound infections.

Zinc

In comparison to the highly developed, complex and possibly not yet fully elucidated iron acquisition system, zinc acquisition seems to be simpler. A high-affinity ABC-type zinc transporter is encoded by the genes *znuABC*, which are regulated by the zinc uptake regulator encoded by the gene *np20*. In this report, the *znuC* was overexpressed in the burn wound infection, and the regulator encoded by *np20* was also upregulated. The regulator *np20* has been reported to be involved in *P. aeruginosa* virulence and PQS signalling (Wang *et al.*, 1996, Gallagher *et al.,* 2002).

In *E. coli* the cellular requirement for zinc is similar as for iron and calcium (Outten & O'Halloran, 2001). The high affinity transport system for zinc ZnuABC was reported to be important for growth of pathogens in the host such as *Salmonella enterica*, *Pasteurella multocida* and *Brucella abortus* (Campoy *et al.*, 2002, Garrido *et al.*, 2003, Kim *et al.*, 2004, Yang *et al.*, 2006, Ammendola *et al.*, 2007).

The difference in the complexity of the machinery for iron and zinc uptake could be due to the elevated zinc levels in the host tissues (Walravens, 1979). However, particularly during the burn injury, the level of the zinc significantly decreases in the wound, while concomitantly increasing its concentration in the urine (Berger *et al.*, 1992). This event, together with a possible zinc limitation strategy employed by the host (Sohnle *et al.*, 1991, Bryant *et al.*, 2004) and overexpression of the eukaryotic matrix metalloprotease (MMP), a zinc containing enzyme involved in wound healing (Woessner, 1991) underscores the hypothesis that *P. aeruginosa* infecting the burn wound has to overcome zinc limitation. Furthermore, many *P. aeruginosa* proteins important for infection are zinc containing such as LasA protease, LasB elastase and present in clinical strains metallo-β-lactamase responsible for resistance to carbapenems. This raises the demand of zinc in the bacterial cells infecting the host.

In addition to the *np20* and *znuC* genes, also the PA3600 and PA3601 genes are overexpressed in the burn wound, lettuce and CF patient infection. As already described in the results section (4.2.1.1.) these genes encode alternative ribosomal proteins which

substitute proteins that would normally bind zinc. This system is believed to be regulated by zinc uptake regulators during the zinc limitation (Owen *et al.,* 2007). These results agree with those reported recently for a CF-sputum analysis, in which *np20* is strongly upregulated (Son et al., 2007). These results altogether lead to the hypothesis that the zinc uptake system may be a possible target for fighting infection.

5.2. Aerobic versus anaerobic respiration

P. aeruginosa cells infecting the host grow in the form of a biofilm (Singh *et al.,* 2000). As a result, nutrient and population gradients develop and *P. aeruginosa* cells in the deeper layers of the biofilm (typically beyond a few hundreds micrometers at most) become exposed to anoxic conditions (Xu *et al.*, 1998). Furthermore, in CF patients and other pulmonary infections, *P. aeruginosa* is embedded in the thickened airway mucus which reinforces these hypoxic gradients.

Under anoxia *P. aeruginosa* is capable of respiration using an inorganic terminal electron acceptor such as nitrate, nitrite or nitric oxide (Hassett *et al.*, 2002). They are also able to ferment arginine via the arginine deaminase pathway, albeit growth is very slow and requires rich medium (Mercenier *et al.*, 1980). In the absence of other sources, *P. aeruginosa* also ferments pyruvate, which is itself not enough for growth, although it enables maintenance of basal metabolism (Eschbach *et al.*, 2004).

As shown in this work, *P. aeruginosa* cells grew abundantly in the mouse tumour, which is essentially anaerobic in deeper compartments (Sutherland, 1988). Genes encoding for enzymes involved in both fermentation strategies, arginine (*arcDABC*) and pyruvate (PA3417, PA3415 and *ackA*) were clearly overexpressed. Furthermore, the genes encoding for the universal stress proteins PA3309 and PA4352 (which are induced by pyruvate fermentation) were upregulated. Pyruvate fermentation is induced only when there is no nitrate and anaerobic oxidation cannot be performed (Eschbach *et al.,* 2004). However, in the tumour mouse infection, the genes responsible for nitrite reduction, *nirSMCDE* and regulator *nir*Q were overexpressed. This possibly reflects the heterogeneity of the sample

from which the RNA was extracted, as it contains a mixture of cells from across the biofilms, both the deep anaerobic and the micro-aerophilic layers of the tumour.

The lettuce infection model had been earlier used to identify those genes responsible for anaerobic growth of *P. aeruginosa* (Filiatrault *et al.,* 2006). However, the transposon mutants of five of the twentyfour genes identified as essential for anaerobic growth on nitrate were attenuated in lettuce infection in this report and none of them were overexpressed in the current work. The genes induced in the lettuce infection model were the global anaerobic regulator Anr and that encoding for the regulatory protein NirQ, as well as the cofactor molybdopterin encoded by the genes *moeA1*, *moeB1*, *moaE* and *moaC*. This makes it difficult to assess whether *P. aeruginosa* cells infecting lettuce are really under anaerobic conditions.

Interestingly, in the burn wound infection it was observed that the *P. aeruginosa* genes responsible for oxidative phosphorylation were mostly downregulated, with the exception of the part of the operon encoding for a succinate dehydrogenase. At the same time, the operon *arcABCD,* encoding for proteins involved in anaerobic arginine fermentation was upregulated. This is somewhat surprising as bacterial growth on burn wounds are not known to contain anaerobic environments, although it may be possible that some deeper layers in superficial biofilms become anoxic. Another reason for the inhibition of oxidative phosphorylation complexes could be due to iron limitation, and more specifically due to a novel, recently reported activity of the Fur regulator (Vasil, 2007, Oglesby *et al.*, 2008). In that work, Vasil and colleagues have shown that Fur, acting via a negative regulation of the sRNA PrrF1 and PrrF2, induces a number of metabolic genes including those encoding enzymes involved in the TCA cycle with succinate dehydrogenase and aconitase B, which may be also involved in anaerobic metabolism. Furthermore, the PrrF1 and PrrF2 regulate periplasmic nitrate reductase. The *acnB* gene (encoding aconitase B) and genes *sdhC* and *sdhD* (encoding for succinate dehydrogenase C and D) were induced in the burn wound infection, however, there was no sign of anaerobic respiration with nitrate reduction. Nevertheless, it is tempting to hypothesise that the aerobic respiration during burn wound

infection is reduced due to limitation of iron, which is a crucial component of the enzymes involved in the oxidative phosphorylation complexes. This in turn, obliges bacterial cells to seek for alternative energy sources, which could be fermentation. Moreover, the anaerobic regulator Anr, which regulates the *arcABCD* operon is not induced during the burn wound infection (as seen on Figure 4-2) therefore, it can be postulated that some alternative regulation occurs, possibly correlated with the iron starvation response.

5.3. Virulence of *P. aeruginosa*

Pathogenicity of *P. aeruginosa* is combinatorial as there are many factors that, jointly, contribute to their virulence. The direct factors are either cell associated such as adhesins, alginate, pili, flagella and lipopolysaccharides, or extracellular such as elastase, exoenzyme S, exotoxin A, hemolysins, iron-binding proteins, leukocidins and proteases. Among the indirect factors, the most important are the availability to overcome stress conditions during host attack such as iron starvation, oxidative stress or the presence of antibacterial compounds. In this work, expression of a number of virulent factors under different conditions was observed. Some of these factors are discussed below.

Alginate production

Alginate production and pathogenicity of *P. aeruginosa* strains is generally associated to conversion of to a mucoid phenotype (Schurr *et al.,* 1996). The expression profiling done here revealed that the genes related to this phenotype were induced during burn wound infection of all three patients. In comparison with the biofilm control, the genes *algU* and *mucA* encoding for sigma and anti-sigma factors were overexpressed. These are important mechanisms for the initiation of alginate production (Boucher *et al.*, 1997, Rowen & Deretic, 2000), which starts with the induction of the gene *algD* encoding GDP-mannose 6-dehydrogenase. The gene *algD* was overexpressed in the burn wound infection in comparison with both controls. The sigma factor *algU* also induces the transcriptional regulator *algR*, which activates positively *algD* and *algC* (overexpressed in burn wound in comparison to planctonic growth) encoding for phosphomannomutase. The regulator AlgR was induced in the burn wound infection, tumour infection and lettuce infection when

compared to the biofilm growth. The AlgR regulator has a more global function, regulating not only the *alg* genes but also inducing the genes encoding type IV fimbrial biogenesis proteins (Lizewski *et al.,* 2004), which were induced in the lettuce infection model. Also the production of hydrogen cyanide and repression of the *rhl* QS system is regulated by AlgR (Morici *et al.*, 2007).

Toxins and secretion systems

Another gene known to be involved in direct virulence is *toxR*, which encodes for the regulator of exotoxin A. This gene was clearly upregulated in the three *P. aeruginosa* strains infecting the respective burn wound patients. Higher levels of expression of exotoxin A are related to low iron concentration and regulation by PvdS (Gaines *et al.*, 2007). The other direct virulence factors secreted during iron starvation were protease IV (gene *piv*) and the insulin-cleaving metalloproteinase (gene *icmP*). The genes of both proteins were upregulated in the burn wound infection, whereas the *piv* gene was induced in the lettuce infection model.

Secretion of various toxins is a major mechanism allowing *P. aeruginosa* to thrive within the infected host. Exotoxin A is secreted via a type II secretion system encoded by genes from the operon *xcp* (Nunn & Lory, 1992). The gene *xcpY* was overexpressed in the lettuce infection. The second secretion system possessed by *P. aeruginosa* is type III secretion system (T3SS), which is involved in direct injection of the effector proteins into the contact host cell (Yahr *et al.*, 1996). This complete system was overexpressed only in the tumour mouse infection. The presence of T3SS is usually recognised as an acute type of infection with high levels of cytotoxity of the infecting *P. aeruginosa* cells (Finck-Barbançon *et al.,* 1997).

It is not clear if the T3SS is induced during prolonged chronic pulmonary infection of CF patients. The report of Lee *et al.*, (2005) has shown that *P. aeruginosa* strains from CF patients isolated shortly after infection and about a decade later, exhibit lower cytotoxity, which may the result from mutations in T3SS apparatus or regulatory networks. On the contrary, burn wound infections are often regarded as acute *P. aeruginosa* infections

(Church *et al.,* 2006). The strains PBCLOp10, PBCLOp11 and PBCLOp17 were isolated from burn wound infections and, accordingly, have a fully active T3SS, which were consistently active in the mouse tumour infection model. However, during the burn wound infection there is no clear overexpression of this system in any of the three strains. This finding, together with other transcriptomic results presented in this thesis, such as the inhibition of oxidative phosporylation (which results in lowered energy production) seem to suggest that burn wound infection represents, in reality, a non-acute state of infection. This may be less surprising than anticipated if one takes into account that the immune system in burn wounds is extremely compromised, thus diminishing the need of bacterial cells to employ such damaging weapons.

Glycine betaine production

The gene *betB* encoding betaine aldehyde dehydrogenase, which transforms betaine aldehyde into glycine betaine was cleary overexpressed, together with its regulator in all of the infection conditions tested in this work; burn wound infection, CF patient infection and lettuce and mouse tumour infection models. This is a very interesting result as the production of glycine betaine is a feature not yet fully understood in connection with virulence. Glycine betaine is an effective osmoprotectant and most likely act as such in *P. aeruginosa* cells growing in the hyperosmotic environment of infected tissues (D'Souza-Ault *et al.*, 1993). The genes from the *bet* pathway can play the dual role of producing the glycine betaine as osmoprotectant but also of utilising its precursors (such as choline and phosphadytylocholin) as carbon and nitrogen sources during infection (Son *et al.,* 2007). Both of these features make the enzyme betaine aldehyde dehydrogenase another potential drug target (Velasco-Garcia *et al.,* 2006).

Quorum sensing

Quorum sensing is the mechanism that allows bacteria to “sense” the density of a bacterial population and to respond to it in an organised manner, regulating thereby a large battery of genes, including those encoding for virulence factors like elastase LasB and rhamnolipid (Schuster & Peter, 2006). The regulon of the QS systems *las* and *rhl* was extensively studied

in vitro. Table 5-1 shows the amount of genes regulated under the different conditions in the work carried out here and which are compared to *in vitro* studies of *las* and *rhl* QS system from the report of Schuster and colleagues, where a wildtype *P. aeruginosa* strain was compared to the double *lasI rhlI* mutant (2003). The results clearly show that a large number of genes reported as QS regulated are both induced and repressed under various infection conditions. In the case of planktonic growth most of the genes are downregulated. These observations underscore the notion that regulation of gene expression is much more complex under *in vivo* than *in vitro* conditions.

Table 5-1: Comparison of QS regulated genes, up and downregulated in different infection settings in this work.

	Biofilm			Planktonic		
	Total differentially regulated	QS regulated up	QS regulated down	Total differentially regulated	QS regulated up	QS regulated down
Burn wound	482	55	37	568	37	81
Tumour	520	27	37	468	9	81
Lettuce	627	50	27	616	10	67
CF patient	-	-	-	73	2	20

The direct effector of the *las* system, elastase LasB, was downregulated in comparison with planktonic growth and did not significantly change in comparison to biofilm growth. The gene *rhlA,* encoding a rhamnosyltransferase involved in rhamnolipid production, was induced in the lettuce infection model and did not change in other conditions tested in this work. The third QS system in *P. aeruginosa*, which is based on PQS signalling molecule, was mainly downregulated under the various conditions, with the highest decrease in expression in the CF infection. This could be due to the high concentration of PQS in the patient's lung (S. Häussler, personal communication).

In the case of the burn wound infection the downregulation of the PQS synthesis pathway may be also due to the regulation of *vfr*, which encodes a virulence factor regulator. The Vfr

activates the ToxR regulator of Exotoxin A production and the synthesis of Protease IV (gene *piv*) (West et al., 1994a), which are both overexpressed in the burn wound infection. Vfr also activates the QS regulator LasR, but has been shown to negatively regulate PQS (Whitchurch *et al.*, 2005). These results show the complexity of gene regulation in various infection conditions and usefulness of direct *in vivo* gene expression profiling in order to reveal these various interplays.

Overall, this work demonstrates that there is likely a sub-set of core genes/regulators that are commonly upregulated across many different infection conditions.

5.4.Real infection settings versus models of infection

Owing the multifactorial and combinatorial nature of *P. aeruginosa*'s virulence, the availability of realistic infection models is crucial for the testing of novel hypotheses regarding both the understanding of its pathogenicity and the development of prevention and therapeutic strategies. Global transcription profiling allowed comparisons between the expression patterns of various *in vivo* and *in vitro* conditions, and among different infection models proposed. The comparison was performed at two levels. Firstly, by comparing the overall global expression patterns (ordination patterns such as MDS) and secondly, by comparing the individual features of virulence present among the different *in vivo* and *in vitro* conditions.

Ordination (using multidimensional scaling) of the global expression profiles obtained from all conditions revealed that, at statistical level, each of the conditions is significantly different from the other. However, the burn wound infection and the tumour infection models are more closely related to each other than to any other condition. The CF patient infection was not considered in the statistical analysis because, apart from being only one isolate from one patient, it was a strain different from that of the three used for the comparative analyses. However, owing to its relevance, the data from this experiment was used for qualitative comparisons. It would certainly be very relevant to continue collecting data on CF to

compare it with the tumour mouse model infection. The relevance of the two infection models tested is discussed briefly below.

Lettuce infection model

Lettuce infection has been used in the past as a model for *P. aeruginosa* infection and the study of QS and anaerobic growth (Filiatrault et al., 2006, Wagner et al., 2007). However, even in these reports only two or three genes proposed to be crucial for either QS or anaerobic growth were shown to be crucial for lettuce infection. The data collected in this study show certain similarities to those reported, namely, the overexpression of QS system *las* or iron starvation response with sigma factor PvdS, but the multivariate statistical analysis shows that the model is far from the other infection conditions and to be closer to the planktonic control growth (see Figure 4-11 and Table 4-7). This shows that the lettuce infection model can be used for the study of particular factors like the already reported QS. However, it is not suitable as a model mimicking mammalian infections by *P. aeruginosa*.

Tumour infection model

The mouse tumour infection model exhibits overexpression of the T3SS together with exotoxins secreted via this system, anaerobic growth in similarity to CF pulmonary infection conditions, heme acquisition system and expression of proteases like PfpL. The gene *cupA1,* which is involved in biofilm formation during lung infection of CF patients was also overexpressed in the tumour. To test this hypothesis, further tests were performed on the *P. aeruginosa* infecting mouse tumour, namely: (i) an histological analysis, which showed that bacteria form a layer, most likely, on the border between anaerobic and microaerofilic compartment of the tumour; (ii) testing the mutants of the genes involved CF patient infection, which revealed that the mutant in gene encoding for anaerobic regulator Anr is highly attenuated and mutants of genes *cupA1* and *pqsA* were much less viable in tumour; and (iii) statistical analysis of transcriptomic data showed that mouse tumour infection is more related to the burn wound infection than non-mammalian lettuce infection or planktonic and biofilm growth. These results altogether suggest the mouse tumour infection model may be a realistic model for the study of *P. aeruginosa* mammalian infections. Further

analysis is needed in order to more accurately appraise in how far it resembles chronic infection.

5.5. The quest for novel anti-bacterial compounds: Protoanemonin

The QS inhibitory potential of protanemonin was tested with the bioassay based on the fluorescent reporter fused with *lasB* promoter. The gene *lasB* is directly regulated by the *las* QS system. The inhibition of the *las* QS system proved the anti-pseudomonal potential of protoanemonin. To further assess the mode of action of this compound, a transcriptomic and proteomic approaches were taken.

The response of *P. aeruginosa* cells to protonamemonin at the transcriptional level showed the differential expression of 84 genes. More than half were repressed. Most of these were QS-regulated. Interestingly, the iron starvation response was induced. Iron starvation is believed to be interconnected with the QS circuit, especially in biofilm grown bacteria (Hentzer *et al.,* 2005), and that PvdS may be AHL-dependent (Juhas *et al.*, 2004). However, previous reports on the QS regulon using transcriptomic approaches have not shown that iron response is coregulated by QS (Hentzer *et al.,* 2003, Schuster *et al.,* 2003, Wagner *et al.,* 2003).

The proteomic data obtained were consistent with transcriptomic results. Among the secreted proteins, downregulation of elastases B (encoded by gene *lasB*) and overexpression of pyoverdine was observed. Only one report, based on a proteomic approach, showed a likely link between QS and iron starvation, where pyoverdine and pyochelin receptors FpvA and FptA were increased in the *lasIrhlI* mutant suggesting that the mutant strains experienced iron limitation despite the presence of excess iron in the medium (Ferro *et al.*, 2003).

This finding shows that the impact and, possibly, the mode of action of protoanemonin, on the *P. aeruginosa* cells differ from furanone C30. The compound furanone C30 did not induce almost any gene in the experimental setting for measurement of QS inhibition. The reason of the induction of iron stress response by protoanemonin needs to be further

investigated in order to elucidate if this is an indirect action via quorum sensing, which is not yet fully understood, or if this compound influences directly the regulation of iron. In either way, the findings show that protoanemonin may be a promising anti-pseudomonal compound.

6. Conclusions and Outlook

In this thesis, the transcriptomic analysis of *P. aeruginosa* in various infection settings was performed. The *in vivo* gene expression was successfully carried out by developing the technical procedures for sample collection, transportation and microarray preparation, which provided a basis to answer the questions stated in the rationale. The main conclusions are thus the following:

- According to the transcriptomic analysis, the main factors underlying burn wound infection by *P. aeruginosa* were iron and zinc acquisition as well as alginate production. The bacterial state during burn wound infection was not fully acute. Bacterial cells undergo serious iron limitation and are slower in metabolism.

- Iron acquisition and alginate production are important mechanisms common among the infection settings studied, namely burn wound, CF patient and tumour model.

- The tumour mouse model is a promising mammalian infection model and, unto a large extent, it mimics the growth conditions of a CF lung. It should be tested further in a wider range of conditions to assess if it can be used as a chronic infection model.

- Plant infection models using lettuce infection may be useful for the study of certain factors such as QS systems, but yielded different results as compared to the real mammalian infections and therefore cannot be used as a reliable infection model.

- The multivariate statistical approach of the global expression data shows that the tumour infection model is the most closely related to the burn wound infection among all conditions. All tested infection and control conditions are statistically different from each other.

- The data analysis techniques, demonstrates that there is a sub-set of core genes that are commonly expressed across many different infection conditions, which shows that the mechanisms of infections are generally common for different conditions.

Conclusions and Outlook

- Global transcriptomic studies revealed that iron acquisition plays a crucial role in the infection by *P. aeruginosa*. Following leads that have shown that protoanemonin inhibited QS in P. aeruginosa, in this thesis, the effect of this compound on the proteomic and transcriptomic profiles of *P. aeruginosa* was tested. It was shown that protoanemonin inhibited QS-related genes and proteins while inducing iron starvation of the cell. The exact mechanism is as yet unknown. This compound thus should be further tested for its potential as anti-infective

- The features observed and the results reported here on the mechanisms and processes used by *P. aeruginosa* during infection provide a wealth of insights that should be explored further in the future. The effect of zinc and the regulation of the zinc response may be a promising new path to understand and combat the virulence of *P. aeruginosa*. Together with the proposed targets like glycine betaine production enzymes, there are a number of hypothetical unknown factors which may play a crucial role in infection. Examples are the proteins encoded by the cluster PA4063-65 and PA4834-37, putatively encoding for a novel siderophore system.

The insights and conclusions obtained in the work presented here provide a foundation for future work directed at the understanding of *P. aeruginosa* infection and finding new prevention and treatment strategies.

7. References

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8. Appendix

Table 8-1: Differentially regulated genes (pfp < 0.05) in burn wound infection in comparison with planktonic and biofilm growth controls.

PA Number	Gene	Fold change compared to Planktonic	Biofilm	Product Name
PA0007		-4.72		hypothetical protein
PA0020		2.23		hypothetical protein
PA0038			3.72	hypothetical protein
PA0050		-2.75	-5.24	hypothetical protein
PA0052		-4.76		hypothetical protein
PA0059	*osmC*	3.70	21.69	osmotically inducible protein OsmC
PA0060		3.56	7.14	conserved hypothetical protein
PA0085			-4.40	conserved hypothetical protein
PA0102		7.46	12.11	probable carbonic anhydrase
PA0103			2.20	probable sulfate transporter
PA0104		2.66	2.75	hypothetical protein
PA0105	*coxB*	-12.23		cytochrome c oxidase, subunit II
PA0106	*coax*	-35.41		cytochrome c oxidase, subunit I
PA0107		-34.07		conserved hypothetical protein
PA0108	*coIII*	-69.43	-3.09	cytochrome c oxidase, subunit III
PA0109		-3.88		hypothetical protein
PA0110		-19.82		hypothetical protein
PA0111		-13.65		hypothetical protein
PA0112		-4.36		hypothetical protein
PA0113		-8.76		probable cytochrome c oxidase assembly factor
PA0122		-46.03	-6.99	conserved hypothetical protein
PA0128	*phnA*		-3.02	conserved hypothetical protein
PA0160			-4.13	hypothetical protein
PA0161			-3.15	hypothetical protein
PA0173		-2.99		probable methylesterase
PA0175	*cheR2*	-10.86		probable chemotaxis protein methyltransferase
PA0176	*aer2*	-8.41		aerotaxis transducer Aer2
PA0177		-8.59		probable purine-binding chemotaxis protein
PA0178		-10.71		probable two-component sensor
PA0179		-26.10		probable two-component response regulator
PA0180		-6.75		probable chemotaxis transducer
PA0215	*madL*	-7.23		probable transporter
PA0249		-3.38		probable acetyltransferase
PA0250		-3.80		conserved hypothetical protein
PA0256		-3.29		hypothetical protein
PA0263	*hcpC*		-54.00	secreted protein Hcp
PA0291	*oprE*		-3.69	Anaerobically-induced outer membrane porin OprE precursor
PA0312		-6.50		conserved hypothetical protein
PA0315			2.55	hypothetical protein
PA0320		3.70	3.64	conserved hypothetical protein
PA0329		-4.10		conserved hypothetical protein
PA0332		-3.35		hypothetical protein
PA0354		3.47		conserved hypothetical protein

PA0355	*pfpI*		7.42	protease PfpI
PA0363	*coaD*		-3.00	phosphopantetheine adenylyltransferase
PA0365		-5.00		hypothetical protein
PA0376	*rpoH*		3.65	sigma factor RpoH
PA0384		-3.81		hypothetical protein
PA0385			-2.88	hypothetical protein
PA0408	*pilG*		-4.09	twitching motility protein PilG
PA0409	*pilH*		-4.59	twitching motility protein PilH
PA0410	*pilI*		-2.65	twitching motility protein PilI
PA0411	*pilJ*		-2.55	twitching motility protein PilJ
PA0423	*pasP*	3.85		PasP
PA0424	*mexR*	2.53		multidrug resistance operon repressor MexR
PA0456			-4.61	probable cold-shock protein
PA0460			2.65	hypothetical protein
PA0472	*fiuI*		3.93	probable sigma-70 factor, ECF subfamily
PA0484		-4.50		conserved hypothetical protein
PA0490			4.87	hypothetical protein
PA0505		-4.34		hypothetical protein
PA0506			-4.00	probable acyl-CoA dehydrogenase
PA0520	*nirQ*	-3.05		regulatory protein NirQ
PA0532		3.87	2.68	hypothetical protein
PA0541			-3.49	hypothetical protein
PA0553			4.07	hypothetical protein
PA0563			-4.39	conserved hypothetical protein
PA0567	*yqaE*		7.62	conserved hypothetical protein
PA0578		9.19	-3.93	conserved hypothetical protein
PA0579	*rpsU*	3.89	-2.82	30S ribosomal protein S21
PA0581	*ygiH*		-3.17	conserved hypothetical protein
PA0586	*ycgB*	-4.22		conserved hypothetical protein
PA0588	*yeaG*		2.43	conserved hypothetical protein
PA0589	*glpE*		-3.51	conserved hypothetical protein
PA0608	*gph*		-2.74	probable phosphoglycolate phosphatase
PA0610	*prtN*	-3.43		transcriptional regulator PrtN
PA0612	*ptrB*	-3.55		repressor, PtrB
PA0623		-2.51		probable bacteriophage protein
PA0624		-4.58		hypothetical protein
PA0652	*vfr*	2.36		transcriptional regulator Vfr
PA0654	*speD*		-3.95	S-adenosylmethionine decarboxylase proenzyme
PA0655			-3.13	hypothetical protein
PA0656	*ycfF*	-4.38		probable HIT family protein
PA0667	*yebA*		-2.84	conserved hypothetical protein
PA0672	*hemO*		6.84	heme oxygenase
PA0707	*toxR*	4.34	4.16	transcriptional regulator ToxR
PA0713			-3.44	hypothetical protein
PA0745		-2.94		probable enoyl-CoA hydratase/isomerase
PA0762	*algU*		2.75	sigma factor AlgU
PA0763	*mucA*		4.78	anti-sigma factor MucA
PA0764	*mucB*	2.61		negative regulator for alginate biosynthesis MucB
PA0766	*mucD*	2.42		serine protease MucD precursor
PA0767	*lepA*		-5.51	GTP-binding protein LepA
PA0768	*lepB*		-3.25	signal peptidase I
PA0778	*icp*	2.31		inhibitor of cysteine peptidase
PA0779		2.33		probable ATP-dependent protease

PA0781		3.14	3.22	hypothetical protein
PA0805			-5.12	hypothetical protein
PA0852	*cbpD*	-3.44		chitin-binding protein CbpD precursor
PA0856		2.73		hypothetical protein
PA0857	*bolA*	2.19		morphogene protein BolA
PA0865	*hpd*	-7.05		4-hydroxyphenylpyruvate dioxygenase
PA0866	*aroP2*	-4.69		aromatic amino acid transport protein AroP2
PA0870	*phhC*	-5.41		aromatic amino acid aminotransferase
PA0871	*phhB*	-4.55		pterin-4-alpha-carbinolamine dehydratase
PA0887	*acsA*		3.73	acetyl-coenzyme A synthetase
PA0890	*aotM*		-3.06	arginine/ornithine transport protein AotM
PA0915	*yehS*		-3.45	conserved hypothetical protein
PA0917	*kup*		-2.57	potassium uptake protein Kup
PA0921		-3.89		hypothetical protein
PA0925		2.44		hypothetical protein
PA0934	*relA*	-2.57		GTP pyrophosphokinase
PA0945	*purM*		-3.18	phosphoribosylaminoimidazole synthetase
PA0952			-3.88	hypothetical protein
PA0954		-3.19		probable acylphosphatase
PA0955			-4.80	hypothetical protein
PA0959		-3.58		hypothetical protein
PA0960		-5.58		hypothetical protein
PA0962			4.25	probable dna-binding stress protein
PA0965	*ruvC*		-4.03	Holliday junction resolvase RuvC
PA0981			-2.53	hypothetical protein
PA0998	*pqsC*		-4.45	Homologous to beta-keto-acyl-acyl-carrier protein synthase
PA1000	*pqsE*		-2.51	Quinolone signal response protein
PA1001	*phnA*	-2.51	-3.69	anthranilate synthase component I
PA1002	*phnB*	-3.72		anthranilate synthase component II
PA1029			2.67	hypothetical protein
PA1034			-2.86	hypothetical protein
PA1041		-33.52		probable outer membrane protein precursor
PA1042		-3.39		conserved hypothetical protein
PA1048		-3.34		probable outer membrane protein precursor
PA1080	*flgE*		-2.67	flagellar hook protein FlgE
PA1092	*fliC*		-3.86	flagellin type B
PA1102	*fliG*	-2.91		flagellar motor switch protein FliG
PA1118			2.62	hypothetical protein
PA1121		-5.21		conserved hypothetical protein
PA1123			-15.42	hypothetical protein
PA1132			-2.87	hypothetical protein
PA1134		3.67	3.69	hypothetical protein
PA1137		3.65	3.92	probable oxidoreductase
PA1151	*imm2*	-2.71		pyocin S2 immunity protein
PA1168		-5.35	-59.91	hypothetical protein
PA1172	*napC*	-4.25		cytochrome c-type protein NapC
PA1173	*napB*	-4.03		cytochrome c-type protein NapB precursor
PA1174	*napA*	-10.94		periplasmic nitrate reductase protein NapA
PA1175	*napD*	-8.01	-2.82	NapD protein of periplasmic nitrate reductase
PA1176	*napF*	-14.21		ferredoxin protein NapF
PA1177	*napE*	-29.71		periplasmic nitrate reductase protein NapE
PA1178	*oprH*	-40.86		PhoP/Q and low Mg2+ inducible outer membrane

				protein H1 precursor
PA1179	*phoP*	-10.24		two-component response regulator PhoP
PA1180	*phoQ*	-5.74		two-component sensor PhoQ
PA1181	*yegE*	-4.98		conserved hypothetical protein
PA1183	*dctA*		-15.66	C4-dicarboxylate transport protein
PA1190	*yohC*	-8.88		conserved hypothetical protein
PA1198			-2.96	conserved hypothetical protein
PA1199			-3.32	probable lipoprotein
PA1202	*ycaC*	-2.80		probable hydrolase
PA1283		-2.90		probable transcriptional regulator
PA1289		-10.93		hypothetical protein
PA1300			5.49	probable sigma-70 factor, ECF subfamily
PA1301			4.22	probable transmembrane sensor
PA1306			-2.63	probable HIT family protein
PA1323			5.48	hypothetical protein
PA1324		3.65	6.73	hypothetical protein
PA1327		-2.77		probable protease
PA1340		-6.04	-4.13	probable permease of ABC transporter
PA1342		-4.83		probable binding protein component of ABC transporter
PA1348		-7.26		hypothetical protein
PA1353		-3.55		hypothetical protein
PA1404		2.41	24.10	hypothetical protein
PA1414		-2.86		hypothetical protein
PA1431	*rsaL*	-6.20		regulatory protein RsaL
PA1439	*ybaN*		-2.61	conserved hypothetical protein
PA1444	*fliN*	-3.48		flagellar motor switch protein FliN
PA1454	*fleN*	-3.06		flagellar synthesis regulator FleN
PA1455	*fliA*	-3.35		sigma factor FliA
PA1456	*cheY*	-3.34		two-component response regulator CheY
PA1458	*cheA*	-2.97		probable two-component sensor
PA1462		-4.28		probable plasmid partitioning protein
PA1464	*cheW*	-2.84		probable purine-binding chemotaxis protein
PA1465		-3.16		hypothetical protein
PA1476	*ccmB*		-2.60	heme exporter protein CcmB
PA1477	*ccmC*		-2.79	heme exporter protein CcmC
PA1478	*ccmD*		-2.99	hypothetical protein
PA1479	*ccmE*		-4.15	cytochrome C-type biogenesis protein CcmE
PA1480	*ccmF*		-4.64	cytochrome C-type biogenesis protein CcmF
PA1482	*ccmH*		-5.22	cytochrome C-type biogenesis protein CcmH
PA1511			-2.56	conserved hypothetical protein
PA1515	*alc*	2.43	2.78	allantoicase
PA1517		2.09	3.78	conserved hypothetical protein
PA1518		4.43	3.61	conserved hypothetical protein
PA1540		2.29		conserved hypothetical protein
PA1543	*apt*		-3.21	adenine phosphoribosyltransferase
PA1545		-3.68		hypothetical protein
PA1546	*hemN*		-3.13	oxygen-independent coproporphyrinogen III oxidase
PA1551	*fixG*		-3.07	probable ferredoxin
PA1555	*ccoP*		-4.30	probable cytochrome c
PA1556	*ccoO*	2.61	-4.92	probable cytochrome c oxidase subunit
PA1557	*ccoN*		-5.60	probable cytochrome oxidase subunit (cbb3-type)
PA1579			2.77	hypothetical protein

PA1581	*sdhC*	3.13		succinate dehydrogenase (C subunit)
PA1582	*sdhD*	3.10		succinate dehydrogenase (D subunit)
PA1584	*sdhB*		-2.59	succinate dehydrogenase (B subunit)
PA1592		2.50		hypothetical protein
PA1596	*htpG*	2.17		heat shock protein HtpG
PA1610	*fabA*		-3.56	beta-hydroxydecanoyl-ACP dehydrase
PA1617		-6.60		probable AMP-binding enzyme
PA1641		-3.68		hypothetical protein
PA1656			-12.65	hypothetical protein
PA1657			-4.36	conserved hypothetical protein
PA1658			-8.61	conserved hypothetical protein
PA1659			-7.13	hypothetical protein
PA1660			-10.06	hypothetical protein
PA1661			-6.99	hypothetical protein
PA1668			-3.07	hypothetical protein
PA1669			-2.69	hypothetical protein
PA1677		-3.08		conserved hypothetical protein
PA1701	*pcr3*		-3.34	conserved hypothetical protein in type III secretion
PA1710	*exsC*	-3.65		ExsC, exoenzyme S synthesis protein C precursor.
PA1713	*exsA*		-3.52	transcriptional regulator ExsA
PA1714	*exsD*		-2.68	ExsD
PA1728		-10.56		hypothetical protein
PA1733		-2.63		conserved hypothetical protein
PA1751		-3.95		hypothetical protein
PA1752		-3.04		hypothetical protein
PA1753		-2.74		conserved hypothetical protein
PA1760		-4.15		probable transcriptional regulator
PA1761		-2.86		hypothetical protein
PA1767			-3.41	hypothetical protein
PA1768		2.01	-2.71	hypothetical protein
PA1775	*cmpX*	2.48		conserved cytoplasmic membrane protein, CmpX protein
PA1784		-7.93		hypothetical protein
PA1787	*acnB*	2.07		aconitate hydratase 2
PA1812	*mltD*	3.36	-3.39	membrane-bound lytic murein transglycosylase D precursor
PA1819	*yjdE*	-2.82		probable amino acid permease
PA1830			-3.78	hypothetical protein
PA1847	*yhgI*	2.45		conserved hypothetical protein
PA1862	*modB*	2.28		molybdenum transport protein ModB
PA1869			-2.81	probable acyl carrier protein
PA1870		3.73	7.84	hypothetical protein
PA1874		-5.13		hypothetical protein
PA1875	*opmL*	-16.57		probable outer membrane protein precursor
PA1876		-3.90		probable ATP-binding/permease fusion ABC transporter
PA1887		-9.08		hypothetical protein
PA1888		-4.95		hypothetical protein
PA1912		2.53	3.23	probable sigma-70 factor, ECF subfamily
PA1914	*hvn*	-5.17		conserved hypothetical protein
PA1931		-2.77	2.59	probable ferredoxin
PA1959	*bacA*	2.57		bacitracin resistance protein
PA1985	*pqqA*		2.61	pyrroloquinoline quinone biosynthesis protein A

PA1988	*pqqD*		3.06	pyrroloquinoline quinone biosynthesis protein D
PA2000	*atoA*	2.39	2.65	probable CoA transferase, subunit B
PA2006		-3.60		probable major facilitator superfamily (MFS) transporter
PA2007	*maiA*	-2.84	6.08	maleylacetoacetate isomerase
PA2008	*fahA*	-3.30		fumarylacetoacetase
PA2015	*liuA*	2.84		putative isovaleryl-CoA dehydrogenase
PA2016	*liuR*	2.96		regulator of liu genes
PA2020	*amrR*	-4.00		probable transcriptional regulator
PA2021			5.66	hypothetical protein
PA2023	*galU*	3.19		UTP--glucose-1-phosphate uridylyltransferase
PA2024		-6.20	3.47	probable ring-cleaving dioxygenase
PA2027		-47.94		hypothetical protein
PA2030		-4.14		hypothetical protein
PA2033			3.03	hypothetical protein
PA2034		-1.29	4.14	hypothetical protein
PA2071	*fusA2*	-2.61		elongation factor G
PA2108		2.88	3.13	probable decarboxylase
PA2134		4.79	30.40	hypothetical protein
PA2135		3.82	6.70	probable transporter
PA2140		3.90	3.50	probable metallothionein
PA2141		5.67	8.02	hypothetical protein
PA2142	*yhxC*	5.00	7.15	probable short-chain dehydrogenase
PA2143		2.33	34.48	hypothetical protein
PA2144	*glgP*	3.35	7.38	glycogen phosphorylase
PA2145		2.35	3.65	hypothetical protein
PA2146	*yciG*	3.40	86.21	conserved hypothetical protein
PA2147	*katE*	3.44	3.37	catalase HPII
PA2148		3.42	7.13	conserved hypothetical protein
PA2149		3.09	8.99	hypothetical protein
PA2150		2.64	2.88	conserved hypothetical protein
PA2151		3.65	4.01	conserved hypothetical protein
PA2152		2.89	5.23	probable trehalose synthase
PA2153	*glgB*	2.60	4.34	1,4-alpha-glucan branching enzyme
PA2157		4.63	5.29	hypothetical protein
PA2158		6.30	14.25	probable alcohol dehydrogenase (Zn-dependent)
PA2159		6.31	52.08	conserved hypothetical protein
PA2160	*glgX*	3.44	4.19	probable glycosyl hydrolase
PA2161		3.14	5.41	hypothetical protein
PA2164		3.73	4.19	probable glycosyl hydrolase
PA2165	*glgA*	4.37	6.33	probable glycogen synthase
PA2166		-5.44	3.92	hypothetical protein
PA2167			3.54	hypothetical protein
PA2168		3.15	5.98	hypothetical protein
PA2169		4.06	22.99	hypothetical protein
PA2170		3.24	32.57	hypothetical protein
PA2171		4.67	18.87	hypothetical protein
PA2172		1.97	7.78	hypothetical protein
PA2173			9.88	hypothetical protein
PA2174			2.61	hypothetical protein
PA2175		2.53	2.99	hypothetical protein
PA2176		2.34	2.69	hypothetical protein
PA2178		3.66	5.45	hypothetical protein

PA2180		4.85	4.97	hypothetical protein
PA2181		3.05	3.92	hypothetical protein
PA2182			3.44	hypothetical protein
PA2183		3.51	5.08	hypothetical protein
PA2184	*yciE*		5.82	conserved hypothetical protein
PA2186		2.14		hypothetical protein
PA2187		2.98	4.56	hypothetical protein
PA2189			2.87	hypothetical protein
PA2190		4.38	44.25	conserved hypothetical protein
PA2192		4.00	7.91	conserved hypothetical protein
PA2199			2.24	probable dehydrogenase
PA2231	*pslA*		-3.28	PslA
PA2233	*pslC*	-3.44		probable glycosyl transferase
PA2240	*pslJ*		-2.54	hypothetical protein
PA2247	*bkdA1*	-4.74		2-oxoisovalerate dehydrogenase (alpha subunit)
PA2248	*bkdA2*	-3.43		2-oxoisovalerate dehydrogenase (beta subunit)
PA2249	*bkdB*	-4.80		branched-chain alpha-keto acid dehydrogenase (lipoamide component)
PA2250	*lpdV*	-7.46		lipoamide dehydrogenase-Val
PA2364		-4.74		hypothetical protein
PA2365		-4.11	-3.29	conserved hypothetical protein
PA2367		-3.38		hypothetical protein
PA2368		-3.11		hypothetical protein
PA2375		-5.10		hypothetical protein
PA2381		-2.79		hypothetical protein
PA2383		2.69	4.24	probable transcriptional regulator
PA2384		3.09	12.29	hypothetical protein
PA2385	*pvdQ*	5.70	5.47	PvdQ
PA2386	*pvdA*	4.16	4.36	L-ornithine N5-oxygenase
PA2393		2.84	3.47	probable dipeptidase precursor
PA2395	*pvdO*	3.79	3.90	PvdO
PA2404		2.94	4.19	hypothetical protein
PA2405		4.01	4.63	hypothetical protein
PA2406		3.51		hypothetical protein
PA2407		2.57	2.50	probable adhesion protein
PA2411		6.05	15.46	probable thioesterase
PA2412		5.76	13.16	conserved hypothetical protein
PA2413	*pvdH*	3.69	4.69	L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase, PvdH
PA2414			7.89	L-sorbosone dehydrogenase
PA2422		-3.04		hypothetical protein
PA2423		-2.70		hypothetical protein
PA2424	*pvdL*	3.23	4.57	PvdL
PA2425	*pvdG*	2.34	3.78	PvdG
PA2426	*pvdS*	5.42	10.43	sigma factor PvdS
PA2433			10.20	hypothetical protein
PA2456			-3.12	hypothetical protein
PA2485		2.07		hypothetical protein
PA2486		4.01	6.33	hypothetical protein
PA2501		-27.00	-4.44	hypothetical protein
PA2504		-6.18		hypothetical protein
PA2531		2.33		probable aminotransferase
PA2562		-3.15		hypothetical protein

PA2564	*tam*	-23.36		hypothetical protein
PA2565		-32.01		hypothetical protein
PA2566		-28.00		conserved hypothetical protein
PA2569			3.15	hypothetical protein
PA2570	*lecA*	-5.73	-3.22	LecA
PA2571		-6.29		probable two-component sensor
PA2573		-4.59		probable chemotaxis transducer
PA2584	*pgsA*		-3.98	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase
PA2591		-9.04	-4.62	probable transcriptional regulator
PA2593			-8.45	hypothetical protein
PA2605	*yheN*	-3.74		conserved hypothetical protein
PA2618		-3.28		hypothetical protein
PA2619	*infA*	2.44	-4.83	initiation factor
PA2620	*clpA*	-3.34		ATP-binding protease component ClpA
PA2621		-3.02		conserved hypothetical protein
PA2622	*cspD*	-8.84	-3.38	cold-shock protein CspD
PA2624	*idh*	5.28		isocitrate dehydrogenase
PA2629	*purB*		-3.89	adenylosuccinate lyase
PA2634	*aceA*	3.44		isocitrate lyase
PA2637	*nuoA*		-3.33	NADH dehydrogenase I chain A
PA2640	*nuoE*	-4.48	-4.59	NADH dehydrogenase I chain E
PA2643	*nuoH*	-5.17	-4.35	NADH dehydrogenase I chain H
PA2646	*nuoK*	-3.44	-3.24	NADH dehydrogenase I chain K
PA2647	*nuoL*	-2.63	-2.53	NADH dehydrogenase I chain L
PA2657			3.05	probable two-component response regulator
PA2658		3.16	8.14	hypothetical protein
PA2659		2.18	3.72	hypothetical protein
PA2666	*ptpS*		-2.82	probable 6-pyruvoyl tetrahydrobiopterin synthase
PA2667		2.33		conserved hypothetical protein
PA2694		2.46	2.73	probable thioredoxin
PA2709	*cysK*		2.38	cysteine synthase A
PA2738	*himA*	-3.38		integration host factor, alpha subunit
PA2741	*rplT*	2.50		50S ribosomal protein L20
PA2746		-10.88		hypothetical protein
PA2747		-4.41		hypothetical protein
PA2748	*mapB*		2.64	probable methionine aminopeptidase
PA2754			5.66	conserved hypothetical protein
PA2755	*eco*	2.27		ecotin precursor
PA2756		-3.37		hypothetical protein
PA2762		-2.74		hypothetical protein
PA2765		-2.55		hypothetical protein
PA2771		-4.29		conserved hypothetical protein
PA2779		-3.70		hypothetical protein
PA2840	*deaD*	2.93	-2.97	probable ATP-dependent RNA helicase
PA2849		-4.24		probable transcriptional regulator
PA2851	*efp*	2.71		translation elongation factor P
PA2883			2.80	hypothetical protein
PA2899		-2.76		probable transcriptional regulator
PA2915		-2.83		hypothetical protein
PA2937		-7.59		hypothetical protein
PA2939	*pepB*	-5.76		probable aminopeptidase
PA2953			-3.39	electron transfer flavoprotein-ubiquinone

				oxidoreductase
PA2955			-2.53	hypothetical protein
PA2957			-3.15	probable transcriptional regulator
PA2966	*acpP*		-3.01	acyl carrier protein
PA2970	*rpmF*	2.17	-2.84	50S ribosomal protein L32
PA2971	*yceD*		-5.10	conserved hypothetical protein
PA2992			-2.68	hypothetical protein
PA3009			-3.26	hypothetical protein
PA3017		-5.04	2.93	conserved hypothetical protein
PA3022		-4.73	-2.83	hypothetical protein
PA3032	*snr1*	-3.95		cytochrome c Snr1
PA3040	*yqjD*		5.96	conserved hypothetical protein
PA3041	*yqjE*		5.00	hypothetical protein
PA3042			5.69	hypothetical protein
PA3049	*rmf*		2.61	ribosome modulation factor
PA3057			-2.70	hypothetical protein
PA3068	*gdhB*	-2.83		NAD-dependent glutamate dehydrogenase
PA3069		2.47		hypothetical protein
PA3096	*xcpY*	-2.88		general secretion pathway protein L
PA3100	*xcpU*	-3.75	-3.10	General secretion pathway outer membrane protein H precursor
PA3101	*xcpT*	-2.92		general secretion pathway protein G
PA3114	*truA*		-2.80	tRNA-pseudouridine synthase I
PA3123		-5.04		conserved hypothetical protein
PA3126	*ibpA*	4.13		heat-shock protein IbpA
PA3186	*oprB*	-6.10		Glucose/carbohydrate outer membrane porin OprB precursor
PA3187	*gltK*	-7.19		probable ATP-binding component of ABC transporter
PA3188	*gltG*	-6.07		probable permease of ABC sugar transporter
PA3189	*gltF*	-3.50		probable permease of ABC sugar transporter
PA3190	*gltB*	-7.27	-5.11	probable binding protein component of ABC sugar transporter
PA3216		-6.71		hypothetical protein
PA3217	*cyaB*		-3.99	CyaB
PA3231			21.65	hypothetical protein
PA3234		-4.54		probable sodium:solute symporter
PA3235		-4.37		conserved hypothetical protein
PA3245	*minE*		-2.81	cell division topological specificity factor MinE
PA3260		-3.20		probable transcriptional regulator
PA3262		2.35		probable peptidyl-prolyl cis-trans isomerase, FkbP-type
PA3266	*capB*	9.76		cold acclimation protein B
PA3274		3.33	13.19	hypothetical protein
PA3278			-3.31	hypothetical protein
PA3280	*oprO*		-2.55	Pyrophosphate-specific outer membrane porin OprO precursor
PA3283		4.49	5.78	conserved hypothetical protein
PA3284		2.58	4.50	hypothetical protein
PA3292			-6.98	hypothetical protein
PA3299	*fadD1*		-2.70	long-chain-fatty-acid--CoA ligase
PA3316		-3.23		probable permease of ABC transporter
PA3326		-4.65		probable Clp-family ATP-dependent protease
PA3332			-4.37	conserved hypothetical protein
PA3343		-2.81		hypothetical protein

PA3345		-2.68		hypothetical protein
PA3346		-6.68		probable two-component response regulator
PA3347		-4.60		hypothetical protein
PA3349		-6.57		probable chemotaxis protein
PA3353		-2.97		hypothetical protein
PA3354		-3.30		hypothetical protein
PA3361	*lecB*	-6.99	-24.70	fucose-binding lectin PA-IIL
PA3385	*amrZ*	-3.64		alginate and motility regulator Z
PA3397	*fpr*	2.93		ferredoxin--NADP+ reductase
PA3407	*hasAp*	31.45	33.33	heme acquisition protein HasAp
PA3415		-6.02		probable dihydrolipoamide acetyltransferase
PA3416		-5.59		probable pyruvate dehydrogenase E1 component, beta chain
PA3417		-4.50		probable pyruvate dehydrogenase E1 component, alpha subunit
PA3418	*ldh*	-9.77		leucine dehydrogenase
PA3431	*ywbG*	10.11	7.82	conserved hypothetical protein
PA3432		6.32	6.66	hypothetical protein
PA3451		-4.51		hypothetical protein
PA3459	*asnB*	4.66	21.98	probable glutamine amidotransferase
PA3460		4.69	13.02	probable acetyltransferase
PA3461	*yhfE*	3.82	20.16	conserved hypothetical protein
PA3465	*yfiS*	-3.37		conserved hypothetical protein
PA3472		2.21		hypothetical protein
PA3477	*rhlR*	-3.15		transcriptional regulator RhlR
PA3489	*rnfA*		-4.99	conserved hypothetical protein
PA3496		-4.21	-5.18	hypothetical protein
PA3520		-4.08	-3.73	hypothetical protein
PA3525	*argG*		-2.68	argininosuccinate synthase
PA3526	*motY*	-2.85		probable outer membrane protein precursor
PA3530	*bfd*		2.67	conserved hypothetical protein
PA3531	*bfrB*		-5.36	bacterioferritin
PA3540	*algD*	3.96	3.90	GDP-mannose 6-dehydrogenase AlgD
PA3568	*ymmS*	-14.46		probable acetyl-coa synthetase
PA3569	*mmsB*	-5.29	2.50	3-hydroxyisobutyrate dehydrogenase
PA3570	*mmsA*	-3.89	2.67	methylmalonate-semialdehyde dehydrogenase
PA3572		-13.37	-2.61	hypothetical protein
PA3576		-3.55		hypothetical protein
PA3584	*glpD*	15.38		glycerol-3-phosphate dehydrogenase
PA3598	*ypqQ*	6.94	6.69	conserved hypothetical protein
PA3600	*rpl36*	434.78	1000.00	conserved hypothetical protein
PA3601	*ykgM*	232.56	175.44	conserved hypothetical protein
PA3611		3.78		hypothetical protein
PA3612	*ypeB*	2.72		conserved hypothetical protein
PA3618	*ygaD*		2.86	conserved hypothetical protein
PA3621	*fdxA*		-5.95	ferredoxin I
PA3622	*rpoS*	-5.33		sigma factor RpoS
PA3635	*eno*	3.75		enolase
PA3645	*fabZ*		-3.13	(3R)-hydroxymyristoyl-[acyl carrier protein] dehydratase
PA3646	*lpxD*		-3.40	UDP-3-O-[3-hydroxylauroyl] glucosamine N-acyltransferase
PA3648			-2.99	probable outer membrane protein precursor

PA3652	*uppS*		-3.02	undecaprenyl pyrophosphate synthetase
PA3653	*frr*		-2.61	ribosome recycling factor
PA3654	*pyrH*		-4.78	uridylate kinase
PA3655	*tsf*	2.60		elongation factor Ts
PA3656	*rpsB*	2.37		30S ribosomal protein S2
PA3662		-9.09		hypothetical protein
PA3684		-31.76	-8.32	hypothetical protein
PA3688		-3.96	2.52	hypothetical protein
PA3691		2.66	16.42	hypothetical protein
PA3692		4.61	14.04	probable outer membrane protein precursor
PA3698		-3.44		hypothetical protein
PA3703	*wspF*	-2.54		probable methylesterase
PA3719		2.31		hypothetical protein
PA3722			-3.82	hypothetical protein
PA3723	*yqjM*	-6.44		probable FMN oxidoreductase
PA3724	*lasB*	-5.68		elastase LasB
PA3731	*yjfJ*	2.32	4.02	conserved hypothetical protein
PA3732	*yjfI*		3.78	conserved hypothetical protein
PA3741			-3.31	hypothetical protein
PA3742	*rplS*	2.28	-4.58	50S ribosomal protein L19
PA3762		2.63	4.21	hypothetical protein
PA3784		-4.41		hypothetical protein
PA3785		-4.32		conserved hypothetical protein
PA3786		-3.35		hypothetical protein
PA3792	*leuA*	-2.87		2-isopropylmalate synthase
PA3795		2.72	4.67	probable oxidoreductase
PA3796		-3.28		hypothetical protein
PA3804			-2.53	hypothetical protein
PA3805	*pilF*		-3.12	type 4 fimbrial biogenesis protein PilF
PA3806	*yfgB*		-2.98	conserved hypothetical protein
PA3809	*fdx2*	2.10		ferredoxin [2Fe-2S]
PA3811	*hscB*	2.32		heat shock protein HscB
PA3812	*iscA*	2.46		probable iron-binding protein IscA
PA3814	*iscS*	4.68		L-cysteine desulfurase (pyridoxal phosphate-dependent)
PA3815		6.89		conserved hypothetical protein
PA3819	*ycfJ*		5.15	conserved hypothetical protein
PA3824	*queA*		-3.64	S-adenosylmethionine:trna ribosyltransferase-isomerase
PA3833		-2.67		hypothetical protein
PA3846		-2.74		hypothetical protein
PA3847		-3.32		conserved hypothetical protein
PA3848		-2.80		hypothetical protein
PA3854		-2.79		hypothetical protein
PA3858	*aapJ*	-7.32		probable amino acid-binding protein
PA3891		2.50	2.62	probable ATP-binding component of ABC transporter
PA3905			-3.50	hypothetical protein
PA3906			-3.90	hypothetical protein
PA3908			-5.14	hypothetical protein
PA3922		-7.91		conserved hypothetical protein
PA3923		-2.87		hypothetical protein
PA3941			-3.05	hypothetical protein
PA3945		-3.10		conserved hypothetical protein

PA3951		3.20	2.47	conserved hypothetical protein
PA3952			2.63	hypothetical protein
PA3957		-6.91		probable short-chain dehydrogenase
PA3966		2.53	-2.99	hypothetical protein
PA3967			-4.90	hypothetical protein
PA3973		-2.69		probable transcriptional regulator
PA3979			-2.98	hypothetical protein
PA3986		-18.49		hypothetical protein
PA4012		-4.05		hypothetical protein
PA4028		2.40		hypothetical protein
PA4031	*ppa*	3.04		inorganic pyrophosphatase
PA4034	*aqpZ*	2.64		aquaporin Z
PA4049		-2.65		hypothetical protein
PA4061	*ybbN*		2.43	probable thioredoxin
PA4063		29.07	24.75	hypothetical protein
PA4064		5.04	4.56	probable ATP-binding component of ABC transporter
PA4065		3.92	3.97	hypothetical protein
PA4079		-2.65		probable dehydrogenase
PA4090		3.15	5.95	hypothetical protein
PA4108		-2.70		hypothetical protein
PA4112		-3.39		probable sensor/response regulator hybrid
PA4129		-9.44	-11.65	hypothetical protein
PA4130		-3.63	-6.63	probable sulfite or nitrite reductase
PA4131			-4.71	probable iron-sulfur protein
PA4132			-4.51	conserved hypothetical protein
PA4133	*ccoN*	-5.09	-12.14	cytochrome c oxidase subunit (cbb3-type)
PA4134		-7.53	-29.56	hypothetical protein
PA4139		-3.29	-26.42	hypothetical protein
PA4140			-7.14	hypothetical protein
PA4141			-3.63	hypothetical protein
PA4142			-6.95	probable secretion protein
PA4170		5.90	6.16	hypothetical protein
PA4171		6.31	26.18	probable protease
PA4172		18.15	23.70	probable nuclease
PA4175	*piv*		5.62	protease IV
PA4218		-1.97		probable transporter
PA4219	*yfpB*	-3.53		hypothetical protein
PA4220	*fptB*	-2.67	7.49	hypothetical protein
PA4221	*fptA*	-1.40	3.72	Fe(III)-pyochelin outer membrane receptor precursor
PA4222	*pchI*	-4.86		probable ATP-binding component of ABC transporter
PA4223	*pchH*	-3.51	2.93	probable ATP-binding component of ABC transporter
PA4224	*pchG*	-3.41	3.83	pyochelin biosynthetic protein PchG
PA4225	*pchF*	-4.28		pyochelin synthetase
PA4226	*pchE*	-3.58		dihydroaeruginoic acid synthetase
PA4227	*pchR*		5.21	transcriptional regulator PchR
PA4228	*pchD*	-3.17		pyochelin biosynthesis protein PchD
PA4229	*pchC*	-4.80		pyochelin biosynthetic protein PchC
PA4230	*pchB*	-1.31	4.32	salicylate biosynthesis protein PchB
PA4231	*pchA*	-3.88		salicylate biosynthesis isochorismate synthase
PA4239	*rpsD*	2.09		30S ribosomal protein S4
PA4240	*rpsK*	3.62		30S ribosomal protein S11
PA4241	*rpsM*	2.60	-3.74	30S ribosomal protein S13
PA4242	*rpmJ*		-3.90	50S ribosomal protein L36

PA4247	*rplR*	2.29		50S ribosomal protein L18
PA4248	*rplF*	2.32		50S ribosomal protein L6
PA4250	*rpsN*		-2.94	30S ribosomal protein S14
PA4251	*rplE*		-2.56	50S ribosomal protein L5
PA4254	*rpsQ*		-2.79	30S ribosomal protein S17
PA4261	*rplW*		-3.01	50S ribosomal protein L23
PA4264	*rpsJ*		-5.12	30S ribosomal protein S10
PA4268	*rpsL*		-2.81	30S ribosomal protein S12
PA4269	*rpoC*		-2.61	DNA-directed RNA polymerase beta* chain
PA4270	*rpoB*		-2.93	DNA-directed RNA polymerase beta chain
PA4271	*rplL*	2.65		50S ribosomal protein L7 / L12
PA4272	*rplJ*	2.59	-2.92	50S ribosomal protein L10
PA4273	*rplA*	2.35		50S ribosomal protein L1
PA4274	*rplK*	2.56	-4.07	50S ribosomal protein L11
PA4275	*nusG*	3.18	-2.62	transcription antitermination protein NusG
PA4276	*secE*	3.16	-2.74	secretion protein SecE
PA4293	*pprA*	-2.87		two-component sensor PprA
PA4294		-10.99		hypothetical protein
PA4296	*pprB*	-13.69		two-component response regulator, PprB
PA4297	*tadG*	-9.63		TadG
PA4298		-9.90		hypothetical protein
PA4299	*tadD*	-5.54		TadD
PA4300	*tadC*	-13.21		TadC
PA4301	*tadB*	-5.59		TadB
PA4302	*tadA*	-20.34		TadA ATPase
PA4303	*tadZ*	-14.24		TadZ
PA4304	*rcpA*	-30.58		RcpA
PA4305	*rcpC*	-32.02		RcpC
PA4306	*flp*	-109.86	-4.40	Type IVb pilin, Flp
PA4317			-3.46	hypothetical protein
PA4318			-2.57	hypothetical protein
PA4324		-3.59	-2.75	hypothetical protein
PA4328		-2.48		hypothetical protein
PA4344			2.73	probable hydrolase
PA4345			4.18	hypothetical protein
PA4352			2.88	conserved hypothetical protein
PA4362		-2.67		hypothetical protein
PA4370	*icmP*		2.92	Insulin-cleaving metalloproteinase outer membrane protein precursor
PA4377		-8.26		hypothetical protein
PA4385	*groEL*	2.18		GroEL protein
PA4390		7.59	6.76	hypothetical protein
PA4394	*yggB*	2.82	6.79	conserved hypothetical protein
PA4405			-3.93	hypothetical protein
PA4419	*ftsL*	-3.68		cell division protein FtsL
PA4424	*yraN*		-2.82	conserved hypothetical protein
PA4426	*yraP*		-2.88	conserved hypothetical protein
PA4427	*sspB*		-8.62	stringent starvation protein B
PA4428	*sspA*		-8.01	stringent starvation protein A
PA4429			-5.23	probable cytochrome c1 precursor
PA4430			-6.78	probable cytochrome b
PA4431			-5.61	probable iron-sulfur protein
PA4432	*rpsI*	2.70	-4.46	30S ribosomal protein S9

PA4433	*rplM*	2.69	-4.51	50S ribosomal protein L13
PA4458	*yrbI*		-2.78	conserved hypothetical protein
PA4459	*yrbK*		-2.64	conserved hypothetical protein
PA4461	*yhbG*	2.21		probable ATP-binding component of ABC transporter
PA4463	*yhbH*	-2.69		conserved hypothetical protein
PA4467			10.06	hypothetical protein
PA4468	*sodM*	3.57	16.95	superoxide dismutase
PA4469			9.95	hypothetical protein
PA4470	*fumC1*		15.70	fumarate hydratase
PA4471	*fagA*	3.22	7.59	hypothetical protein
PA4474	*tldD*		2.48	conserved hypothetical protein
PA4479	*mreD*	3.12		rod shape-determining protein MreD
PA4480	*mreC*		-3.44	rod shape-determining protein MreC
PA4481	*mreB*	5.87		rod shape-determining protein MreB
PA4494			-4.60	probable two-component sensor
PA4495			2.52	hypothetical protein
PA4507		-9.29		hypothetical protein
PA4528	*pilD*		-3.14	type 4 prepilin peptidase PilD
PA4530			-2.71	conserved hypothetical protein
PA4542	*clpB*	4.66	4.30	ClpB protein
PA4563	*rpsT*	5.19	-11.03	30S ribosomal protein S20
PA4565	*proB*		-2.91	glutamate 5-kinase
PA4567	*rpmA*		-3.54	50S ribosomal protein L27
PA4568	*rplU*	2.63	-2.57	50S ribosomal protein L21
PA4569	*ispB*		-2.56	octaprenyl-diphosphate synthase
PA4570			14.01	hypothetical protein
PA4572	*fklB*	-8.69		peptidyl-prolyl cis-trans isomerase FklB
PA4573		-27.56		hypothetical protein
PA4575			2.45	hypothetical protein
PA4590	*pra*	-8.54		protein activator
PA4602	*glyA3*		-4.94	serine hydroxymethyltransferase
PA4606	*cstA*	-3.23		conserved hypothetical protein
PA4607		-10.48		hypothetical protein
PA4608		-10.60		hypothetical protein
PA4611		-4.63		hypothetical protein
PA4625			-4.78	hypothetical protein
PA4633		-2.75		probable chemotaxis transducer
PA4641		-4.80		still frameshift hypothetical protein
PA4648		-25.57		hypothetical protein
PA4651		-3.14		probable pili assembly chaperone
PA4657			3.67	hypothetical protein
PA4661	*pagL*		3.11	Lipid A 3-O-deacylase
PA4670	*prs*	7.79	-2.86	ribose-phosphate pyrophosphokinase
PA4671	*rplY*	3.05		probable ribosomal protein L25
PA4672	*pth*		-6.47	peptidyl-tRNA hydrolase
PA4673	*ychF*	2.59	-3.14	conserved hypothetical protein
PA4678	*rimI*		-2.63	peptide n-acetyltransferase RimI
PA4702		-2.93		hypothetical protein
PA4703		-4.24		hypothetical protein
PA4713		-2.58		hypothetical protein
PA4717		-4.53		conserved hypothetical protein
PA4733	*acsB*	-2.63		acetyl-coenzyme A synthetase
PA4735		-2.57		hypothetical protein

PA4736		-3.22		hypothetical protein
PA4741	*rpsO*		-3.28	30S ribosomal protein S15
PA4743	*rbfA*		-5.34	ribosome-binding factor A
PA4745	*nusA*	2.37		N utilization substance protein A
PA4746	*yhbC*	4.76	-4.12	conserved hypothetical protein
PA4747	*secG*		-3.64	secretion protein SecG
PA4753	*yhbY*		-3.74	conserved hypothetical protein
PA4757	*yeaS*		-3.44	conserved hypothetical protein
PA4759	*dapB*	3.05		dihydrodipicolinate reductase
PA4761	*dnaK*	4.07	3.57	DnaK protein
PA4765	*omlA*	2.08	-2.88	Outer membrane lipoprotein OmlA precursor
PA4767	*yfjG*	-3.62		conserved hypothetical protein
PA4768	*smpB*		-3.32	SmpB protein
PA4778	*ybbI*	-3.47		probable transcriptional regulator
PA4780		-2.75		conserved hypothetical protein
PA4781		-11.59		probable two-component response regulator
PA4782		-4.19		hypothetical protein
PA4787		-5.85		probable transcriptional regulator
PA4810	*fdnI*	-2.91		nitrate-inducible formate dehydrogenase, gamma subunit
PA4811	*fdnH*	-4.66		nitrate-inducible formate dehydrogenase, beta subunit
PA4812	*fdnG*	-3.33		formate dehydrogenase-O, major subunit
PA4833			5.77	conserved hypothetical protein
PA4834		6.45	6.07	hypothetical protein
PA4835		14.31	13.40	hypothetical protein
PA4836		22.42	20.24	hypothetical protein
PA4837		24.15	22.17	probable outer membrane protein precursor
PA4838		3.91	3.92	hypothetical protein
PA4842			3.26	hypothetical protein
PA4846	*aroQ1*		-2.83	3-dehydroquinate dehydratase
PA4853	*fis*		-4.14	DNA-binding protein Fis
PA4854	*purH*		-2.80	phosphoribosylaminoimidazolecarboxamide formyltransferase
PA4874	*psiF*	-3.00		conserved hypothetical protein
PA4876	*osmE*		5.56	osmotically inducible lipoprotein OsmE
PA4877			8.05	hypothetical protein
PA4896		3.43	3.54	probable sigma-70 factor, ECF subfamily
PA4913		-4.23		probable binding protein component of ABC transporter
PA4915		-5.62		probable chemotaxis transducer
PA4925		-9.23		conserved hypothetical protein
PA4929		-11.81		hypothetical protein
PA4932	*rplI*	3.47		50S ribosomal protein L9
PA4934	*rpsR*		-2.72	30S ribosomal protein S18
PA4935	*rpsF*	2.64	-2.53	30S ribosomal protein S6
PA4936	*spoU*		2.68	probable rRNA methylase
PA4940	*yjeT*		-3.88	conserved hypothetical protein
PA4947	*amiB*	-3.42		N-acetylmuramoyl-L-alanine amidase
PA4962	*ybcI*		-3.12	conserved hypothetical protein
PA4971	*aspP*		3.01	adenosine diphosphate sugar pyrophosphatase
PA5030	*ynfM*	4.26		probable major facilitator superfamily (MFS) transporter
PA5033			-2.99	hypothetical protein

PA5040	*pilQ*		-2.95	Type 4 fimbrial biogenesis outer membrane protein PilQ precursor
PA5041	*pilP*	-2.84	-5.53	type 4 fimbrial biogenesis protein PilP
PA5042	*pilO*	-2.92	-4.28	type 4 fimbrial biogenesis protein PilO
PA5045	*ponA*		-2.63	penicillin-binding protein 1A
PA5049	*rpmE*		-7.40	50S ribosomal protein L31
PA5053	*hslV*	2.35		heat shock protein HslV
PA5054	*hslU*	3.34		heat shock protein HslU
PA5055		2.54		hypothetical protein
PA5058	*phaC2*	-5.36		poly(3-hydroxyalkanoic acid) synthase 2
PA5060	*phaF*		3.05	polyhydroxyalkanoate synthesis protein PhaF
PA5101		-7.40	-2.51	hypothetical protein
PA5111	*gloA3*		2.69	lactoylglutathione lyase
PA5117	*typA*	2.40	-3.33	regulatory protein TypA
PA5118	*thiI*		-3.94	thiazole biosynthesis protein ThiI
PA5128	*secB*	2.57		secretion protein SecB
PA5129	*grx*	4.28		glutaredoxin
PA5139			-3.65	hypothetical protein
PA5150			3.58	probable short-chain dehydrogenase
PA5153		-3.73		probable periplasmic binding protein
PA5170	*arcD*		3.84	arginine/ornithine antiporter
PA5171	*arcA*		3.65	arginine deiminase
PA5172	*arcB*		3.60	ornithine carbamoyltransferase, catabolic
PA5173	*arcC*		4.14	carbamate kinase
PA5194		2.08		hypothetical protein
PA5200	*ompR*		2.83	two-component response regulator OmpR
PA5208		-5.37		conserved hypothetical protein
PA5212			5.31	hypothetical protein
PA5213	*gcvP1*	-4.78		glycine cleavage system protein P1
PA5214	*gcvH1*	-2.49		glycine cleavage system protein H1
PA5217			2.71	probable binding protein component of ABC iron transporter
PA5239	*rho*	3.79	-2.95	transcription termination factor Rho
PA5255	*algQ*	-3.98		Alginate regulatory protein AlgQ
PA5261	*algR*	-3.10	3.68	alginate biosynthesis regulatory protein AlgR
PA5288	*glnK*	-5.19		nitrogen regulatory protein P-II 2
PA5296	*rep*		-2.76	ATP-dependent DNA helicase Rep
PA5298	*xpt ;*	3.66		xanthine phosphoribosyltransferase
PA5300	*cycB*		-4.16	cytochrome c5
PA5301	*ycjC*	-2.93		probable transcriptional regulator
PA5315	*rpmG*	2.15	-6.43	50S ribosomal protein L33
PA5316	*rpmB*		-4.34	50S ribosomal protein L28
PA5322	*algC*	2.73		phosphomannomutase AlgC
PA5338	*spoT*	2.19		guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase
PA5340		3.50		hypothetical protein
PA5348		-16.26		probable DNA-binding protein
PA5350	*rubA2*	-4.04		Rubredoxin 2
PA5351	*rubA1*		-3.40	Rubredoxin 1
PA5359		-3.55		hypothetical protein
PA5366	*pstB*		-2.82	ATP-binding component of ABC phosphate transporter
PA5367	*pstA*		-3.70	membrane protein component of ABC phosphate transporter

PA5369	*pstS*		-2.77	phosphate ABC transporter, periplasmic phosphate-binding protein, PstS
PA5373	*betB*	4.85	3.87	betaine aldehyde dehydrogenase
PA5374	*betI*	28.01	7.17	transcriptional regulator BetI
PA5380	*gbdR*	-3.79		GbdR
PA5388		13.68	13.16	hypothetical protein
PA5408			2.93	hypothetical protein
PA5409		-3.66		hypothetical protein
PA5424	*yeaQ ;*	-3.00		conserved hypothetical protein
PA5429	*aspA*		-1.68	aspartate ammonia-lyase
PA5479	*gltP*	2.97	-2.84	proton-glutamate symporter
PA5481			9.56	hypothetical protein
PA5482			8.40	hypothetical protein
PA5490	*cc4*		-6.53	cytochrome c4 precursor
PA5491			-3.65	probable cytochrome
PA5492			-2.55	conserved hypothetical protein
PA5494		-2.74		hypothetical protein
PA5499	*np20*	4.18	5.68	transcriptional regulator np20
PA5500	*znuC*	2.39		zinc transport protein ZnuC
PA5504			-2.92	probable permease of ABC transporter
PA5505			-2.97	probable TonB-dependent receptor
PA5527		-6.15		hypothetical protein
PA5531	*tonB*		3.06	TonB protein
PA5534		2.49		hypothetical protein
PA5535		9.98	10.28	conserved hypothetical protein
PA5536		6.45	7.07	conserved hypothetical protein
PA5537		2.86	4.07	hypothetical protein
PA5538	*amiA*	5.27	5.38	N-acetylmuramoyl-L-alanine amidase
PA5539		5.12	5.20	hypothetical protein
PA5540		18.35	16.45	hypothetical protein
PA5541	*pyrQ*	11.20	10.70	dihydroorotase
PA5543		-3.49		hypothetical protein
PA5546		-2.59	2.86	conserved hypothetical protein
PA5553	*atpC*		-2.81	ATP synthase epsilon chain
PA5555	*atpG*		-2.95	ATP synthase gamma chain
PA5560	*atpB*	2.36	-3.10	ATP synthase A chain
PA5568		2.52	-4.05	conserved hypothetical protein
PA5569	*rnpA*	2.39		ribonuclease P protein component
AF035937cds6	*wbpO*		-2.91	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF035937cds8	*wbpQ*		-2.48	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF241171cds39			2.29	first half of finger motif
Pae_orfC_at		-2.92		putative 3-oxoacyl (acyl carrier protein) synthase - fatty acid biosnthesis, from glycosylation island genes in strain PAK
Pae_tRNA_Ala			-6.75	tRNA_Alanine
Pae_tRNA_Arg		2.77	6.64	tRNA_Arginine
Pae_tRNA_Asn			-2.86	tRNA_Asparagine
Pae_tRNA_Gln			-4.79	tRNA_Glutamine
Pae_tRNA_Gly			-7.38	tRNA_Glycine
Pae_tRNA_His			-4.34	tRNA_Histidine
Pae_tRNA_Ile			-5.32	tRNA_Isoleucine

Pae_tRNA_Leu		-2.83	tRNA_Leucine
Pae_tRNA_Lys	2.12		tRNA_Lysine
Pae_tRNA_Ser	-10.41		tRNA_Serine
Pae_tRNA_Trp		-3.73	tRNA_Tryptophan
Pae_tRNA_Tyr		-3.32	tRNA_Tyrosine

Table 8-2: Differentially regulated genes (pfp < 0.05) in mouse tumour infection in comparison with planktonic and biofilm growth controls.

PA Number	Gene	Fold change compared to Planktonic	Biofilm	Product Name
PA0001	*dnaA*		-3.12	chromosomal replication initiator protein DnaA
PA0020		3.27		hypothetical protein
PA0044	*exoT*	6.51	10.52	exoenzyme T
PA0059	*osmC*		3.57	osmotically inducible protein OsmC
PA0061			3.23	hypothetical protein
PA0067	*prlC*		2.83	oligopeptidase A
PA0093		2.38	2.83	hypothetical protein
PA0102		3.96	6.44	probable carbonic anhydrase
PA0122		-45.85	-6.96	conserved hypothetical protein
PA0128	*phnA*		-3.31	conserved hypothetical protein
PA0130		2.45	2.96	probable aldehyde dehydrogenase
PA0132	*oapT*	8.00	6.51	beta-alanine--pyruvate transaminase
PA0141		4.16	3.04	conserved hypothetical protein
PA0145		2.49		hypothetical protein
PA0160			-4.27	hypothetical protein
PA0161			-4.99	hypothetical protein
PA0168	*yigZ*	2.48		conserved hypothetical protein
PA0179		-69.65	-3.59	probable two-component response regulator
PA0197		2.63	2.37	hypothetical protein
PA0200			10.05	hypothetical protein
PA0257			-2.39	hypothetical protein
PA0263	*hcpC*		-74.45	secreted protein Hcp
PA0271			2.44	hypothetical protein
PA0276		5.47	3.51	hypothetical protein
PA0291	*oprE*		-2.81	Anaerobically-induced outer membrane porin OprE precursor
PA0297	*spuA*		-3.19	probable glutamine amidotransferase
PA0312		-3.33	2.67	conserved hypothetical protein
PA0320		3.22	3.17	conserved hypothetical protein
PA0332			3.97	hypothetical protein
PA0355	*pfpI*		3.12	protease PfpI
PA0363	*coaD*		-3.09	phosphopantetheine adenylyltransferase
PA0385			-3.92	hypothetical protein
PA0388			4.55	hypothetical protein
PA0408	*pilG*		-3.66	twitching motility protein PilG
PA0409	*pilH*		-3.78	twitching motility protein PilH
PA0410	*pilI*		-3.15	twitching motility protein PilI
PA0415	*chpC*	2.61	2.59	probable chemotaxis protein
PA0424	*mexR*		-5.69	multidrug resistance operon repressor MexR
PA0432	*sahH*		-3.25	S-adenosyl-L-homocysteine hydrolase
PA0433		2.43	2.51	hypothetical protein
PA0447	*gcdH*		-4.34	glutaryl-CoA dehydrogenase
PA0456			-14.44	probable cold-shock protein

PA0505		-15.99	-2.86	hypothetical protein
PA0506		2.53		probable acyl-CoA dehydrogenase
PA0510	*nirE*	4.12	3.24	probable uroporphyrin-III c-methyltransferase
PA0515	*nirD*	4.63		probable transcriptional regulator
PA0517	*nirC*	3.53		probable c-type cytochrome precursor
PA0518	*nirM*	13.97	7.77	cytochrome c-551 precursor
PA0519	*nirS*	6.35	8.83	nitrite reductase precursor
PA0520	*nirQ*		4.14	regulatory protein NirQ
PA0526		2.58	3.31	hypothetical protein
PA0541			-2.55	hypothetical protein
PA0545		6.83	8.39	hypothetical protein
PA0546	*metK*		-5.17	methionine adenosyltransferase
PA0563			-4.60	conserved hypothetical protein
PA0572		2.80		hypothetical protein
PA0576	*rpoD*		-7.08	sigma factor RpoD
PA0578			-28.77	conserved hypothetical protein
PA0579	*rpsU*		-14.96	30S ribosomal protein S21
PA0581	*ygiH*		-3.88	conserved hypothetical protein
PA0589	*glpE*		-3.52	conserved hypothetical protein
PA0595	*ostA*		-3.96	organic solvent tolerance protein OstA precursor
PA0610	*prtN*		3.94	transcriptional regulator PrtN
PA0614			2.81	hypothetical protein
PA0647			2.92	hypothetical protein
PA0654	*speD*		-4.30	S-adenosylmethionine decarboxylase proenzyme
PA0655			-2.55	hypothetical protein
PA0665	*yadR*		-3.66	conserved hypothetical protein
PA0667	*yebA*		-4.74	conserved hypothetical protein
PA0713		9.69	7.63	hypothetical protein
PA0747			3.19	probable aldehyde dehydrogenase
PA0767	*lepA*		-10.50	GTP-binding protein LepA
PA0768	*lepB*		-7.95	signal peptidase I
PA0781		3.02	3.09	hypothetical protein
PA0805		-7.83	-41.61	hypothetical protein
PA0836	*ackA*	3.32	5.03	acetate kinase
PA0864		3.37	3.27	probable transcriptional regulator
PA0890	*aotM*		-3.96	arginine/ornithine transport protein AotM
PA0915	*yehS*		-3.48	conserved hypothetical protein
PA0916	*yliG*		-2.48	conserved hypothetical protein
PA0929	*pirR*	-5.61	-2.66	two-component response regulator
PA0936	*lpxO2*		-3.22	lipopolysaccharide biosynthetic protein LpxO2
PA0942			4.18	probable transcriptional regulator
PA0944	*purN*		-3.31	phosphoribosylaminoimidazole synthetase
PA0945	*purM*		-5.08	phosphoribosylaminoimidazole synthetase
PA0952		7.69		hypothetical protein
PA0955			-5.13	hypothetical protein
PA0962			3.99	probable dna-binding stress protein
PA0965	*ruvC*		-3.43	Holliday junction resolvase RuvC
PA0969	*tolQ*		-4.25	TolQ protein
PA0974			-5.73	conserved hypothetical protein
PA0984		2.34		colicin immunity protein
PA0996	*pqsA*	-3.71	-5.30	probable coenzyme A ligase
PA0998	*pqsC*	-3.41	-5.01	Homologous to beta-keto-acyl-acyl-carrier protein synthase

PA1000	*pqsE*	-2.54	-2.72	Quinolone signal response protein
PA1001	*phnA*		-3.07	anthranilate synthase component I
PA1006	*yrkI*		-3.21	conserved hypothetical protein
PA1029		11.24	29.33	hypothetical protein
PA1030			2.99	hypothetical protein
PA1034			-3.69	hypothetical protein
PA1077	*flgB*		-3.00	flagellar basal-body rod protein FlgB
PA1080	*flgE*		-3.19	flagellar hook protein FlgE
PA1118			2.71	hypothetical protein
PA1123			-13.29	hypothetical protein
PA1126		2.67		hypothetical protein
PA1127	*gsp69*		3.24	probable oxidoreductase
PA1131			-2.30	probable major facilitator superfamily (MFS) transporter
PA1132			-2.88	hypothetical protein
PA1151	*imm2*	-9.51	-6.17	pyocin S2 immunity protein
PA1168		-3.98	-44.61	hypothetical protein
PA1177	*napE*	-7.98	5.95	periplasmic nitrate reductase protein NapE
PA1183	*dctA*		-16.72	C4-dicarboxylate transport protein
PA1193			-3.12	hypothetical protein
PA1195		9.06	7.13	hypothetical protein
PA1199			-4.32	probable lipoprotein
PA1305		-5.71	-13.65	hypothetical protein
PA1306			-2.66	probable HIT family protein
PA1323			3.78	hypothetical protein
PA1337	*ansB*		2.54	glutaminase-asparaginase
PA1340		-5.08	-3.47	probable permease of ABC transporter
PA1388		2.29	2.82	hypothetical protein
PA1404			3.98	hypothetical protein
PA1414			7.30	hypothetical protein
PA1429		3.86	4.03	probable cation-transporting P-type ATPase
PA1431	*rsaL*	-11.28	-4.03	regulatory protein RsaL
PA1439	*ybaN*		-4.20	conserved hypothetical protein
PA1457	*cheZ*	-5.42	-3.47	chemotaxis protein CheZ
PA1477	*ccmC*		-4.28	heme exporter protein CcmC
PA1479	*ccmE*		-3.66	cytochrome C-type biogenesis protein CcmE
PA1480	*ccmF*		-2.55	cytochrome C-type biogenesis protein CcmF
PA1482	*ccmH*		-6.01	cytochrome C-type biogenesis protein CcmH
PA1493	*cysP*	-3.97	-4.21	sulfate-binding protein of ABC transporter
PA1506		2.23		hypothetical protein
PA1517			3.85	conserved hypothetical protein
PA1533		-3.49	-3.07	conserved hypothetical protein
PA1540			-2.41	conserved hypothetical protein
PA1543	*apt*		-4.69	adenine phosphoribosyltransferase
PA1544	*anr*		-4.74	transcriptional regulator Anr
PA1546	*hemN*	2.36		oxygen-independent coproporphyrinogen III oxidase
PA1553	*fixO*	-3.32	-5.48	probable cytochrome c oxidase subunit
PA1555	*ccoP*	17.06		probable cytochrome c
PA1556	*ccoO*	5.68		probable cytochrome c oxidase subunit
PA1557	*ccoN*	11.35		probable cytochrome oxidase subunit (cbb3-type)
PA1564			-5.26	conserved hypothetical protein
PA1580	*gltA*		-3.86	citrate synthase
PA1581	*sdhC*		-4.38	succinate dehydrogenase (C subunit)

PA1583	*sdhA*		-3.31	succinate dehydrogenase (A subunit)
PA1584	*sdhB*		-6.58	succinate dehydrogenase (B subunit)
PA1610	*fabA*	-5.81	-23.50	beta-hydroxydecanoyl-ACP dehydrase
PA1656			-11.00	hypothetical protein
PA1657			-3.36	conserved hypothetical protein
PA1658			-6.27	conserved hypothetical protein
PA1659			-5.27	hypothetical protein
PA1660			-8.35	hypothetical protein
PA1661			-6.42	hypothetical protein
PA1662		2.27		probable ClpA/B-type protease
PA1673		4.00	7.12	hypothetical protein
PA1692	*pscS*	2.46		probable translocation protein in type III secretion
PA1695	*pscP*	2.93	2.81	translocation protein in type III secretion
PA1696	*pscO*	3.22		translocation protein in type III secretion
PA1699	*pcr1*	4.04		conserved hypothetical protein in type III secretion
PA1700	*pcr2*	8.82	5.61	conserved hypothetical protein in type III secretion
PA1701	*pcr3*	5.95		conserved hypothetical protein in type III secretion
PA1704	*pcrR*	2.08	3.11	transcriptional regulator protein PcrR
PA1706	*pcrV*	6.69	8.19	type III secretion protein PcrV
PA1707	*pcrH*	7.03	7.28	regulatory protein PcrH
PA1708	*popB*	5.57	7.43	translocator protein PopB
PA1709	*popD*	5.75	7.14	Translocator outer membrane protein PopD precursor
PA1711	*exsE*	4.26	5.81	ExsE
PA1712	*exsB*	2.76		exoenzyme S synthesis protein B
PA1714	*exsD*	5.52		ExsD
PA1715	*pscB*	7.81	4.80	type III export apparatus protein
PA1716	*pscC*	4.98		Type III secretion outer membrane protein PscC precursor
PA1717	*pscD*	5.64	5.47	type III export protein PscD
PA1718	*pscE*	11.83	6.06	type III export protein PscE
PA1719	*pscF*	4.32	2.96	type III export protein PscF
PA1720	*pscG*	3.94	3.58	type III export protein PscG
PA1721	*pscH*	4.13	4.14	type III export protein PscH
PA1722	*pscI*	4.61	4.38	type III export protein PscI
PA1723	*pscJ*	2.59		type III export protein PscJ
PA1724	*pscK*	2.41		type III export protein PscK
PA1728		-16.46	1.47	hypothetical protein
PA1734		2.11		hypothetical protein
PA1742		3.09	3.29	probable amidotransferase
PA1746		11.07	15.29	hypothetical protein
PA1767			-4.50	hypothetical protein
PA1768			-4.45	hypothetical protein
PA1774	*cfrX*	-3.01	-8.29	CfrX protein
PA1776	*sigX*	-4.29	-3.42	ECF sigma factor SigX
PA1789			3.23	hypothetical protein
PA1801	*clpP*		-3.38	ATP-dependent Clp protease proteolytic subunit
PA1812	*mltD*		-7.87	membrane-bound lytic murein transglycosylase D precursor
PA1833	*yhfP*		3.49	probable oxidoreductase
PA1847	*yhgI*		-3.39	conserved hypothetical protein
PA1852		-9.58	-17.22	hypothetical protein
PA1869			-3.63	probable acyl carrier protein

PA1879		3.02		hypothetical protein
PA1925		2.51		hypothetical protein
PA2007	*maiA*	-5.30	3.26	maleylacetoacetate isomerase
PA2021			3.71	hypothetical protein
PA2031			3.15	hypothetical protein
PA2042	*ygjU*		-2.76	probable transporter (membrane subunit)
PA2119	*adh*	3.28	2.95	alcohol dehydrogenase (Zn-dependent)
PA2127		4.14		conserved hypothetical protein
PA2128	*cupA1*	16.61	10.25	fimbrial subunit CupA1
PA2134			2.81	hypothetical protein
PA2143			4.68	hypothetical protein
PA2146	*yciG*	-6.54	3.86	conserved hypothetical protein
PA2168			2.61	hypothetical protein
PA2169			3.26	hypothetical protein
PA2170			4.40	hypothetical protein
PA2171			5.53	hypothetical protein
PA2172			3.26	hypothetical protein
PA2173			4.87	hypothetical protein
PA2190			7.45	conserved hypothetical protein
PA2191	*exoY*	4.19	4.18	adenylate cyclase ExoY
PA2231	*pslA*		-3.67	PslA
PA2242	*pslL*		-3.62	hypothetical protein
PA2249	*bkdB*	-3.04	3.30	branched-chain alpha-keto acid dehydrogenase (lipoamide component)
PA2273	*soxR*		2.59	probable transcriptional regulator
PA2321	*gntV*		-3.50	gluconokinase
PA2365		-5.40	-4.32	conserved hypothetical protein
PA2392	*pvdP*	4.02	4.41	PvdP
PA2411			3.26	probable thioesterase
PA2412			3.29	conserved hypothetical protein
PA2436		3.00	2.78	hypothetical protein
PA2442	*gcvT2*		3.24	glycine cleavage system protein T2
PA2453		4.24		hypothetical protein
PA2460		2.36	2.41	hypothetical protein
PA2573			2.93	probable chemotaxis transducer
PA2576		2.46	2.90	hypothetical protein
PA2584	*pgsA*		-6.05	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase
PA2591		-16.12	-8.25	probable transcriptional regulator
PA2592	*potF5*		-3.05	probable periplasmic spermidine/putrescine-binding protein
PA2593			-8.91	hypothetical protein
PA2619	*infA*		-15.58	initiation factor
PA2627	*ycfC*		-3.37	conserved hypothetical protein
PA2629	*purB*		-3.89	adenylosuccinate lyase
PA2637	*nuoA*		-4.26	NADH dehydrogenase I chain A
PA2640	*nuoE*	-3.80	-3.89	NADH dehydrogenase I chain E
PA2643	*nuoH*	-5.10	-4.30	NADH dehydrogenase I chain H
PA2658			3.92	hypothetical protein
PA2659		1.66	2.83	hypothetical protein
PA2662		3.26	3.16	conserved hypothetical protein
PA2709	*cysK*		2.93	cysteine synthase A
PA2741	*rplT*		-4.62	50S ribosomal protein L20

PA2753		4.22	4.40	hypothetical protein
PA2754			5.78	conserved hypothetical protein
PA2769			-3.48	hypothetical protein
PA2797		-8.21	-8.84	hypothetical protein
PA2826			4.04	probable glutathione peroxidase
PA2827	*yeaA*		3.00	conserved hypothetical protein
PA2840	*deaD*		-5.68	probable ATP-dependent RNA helicase
PA2851	*efp*		-4.74	translation elongation factor P
PA2864		-3.95	-4.50	conserved hypothetical protein
PA2899		-4.82	-2.53	probable transcriptional regulator
PA2931		7.45	9.59	probable transcriptional regulator
PA2957			-4.68	probable transcriptional regulator
PA2960	*pilZ*		-2.54	type 4 fimbrial biogenesis protein PilZ
PA2966	*acpP*		-8.93	acyl carrier protein
PA2970	*rpmF*		-11.43	50S ribosomal protein L32
PA2971	*yceD*		-7.80	conserved hypothetical protein
PA3006	*psrA*	2.29		transcriptional regulator PsrA
PA3009			-8.62	hypothetical protein
PA3017		-4.21	3.51	conserved hypothetical protein
PA3031			-5.53	hypothetical protein
PA3040	*yqjD*		5.90	conserved hypothetical protein
PA3041	*yqjE*		3.36	hypothetical protein
PA3056		2.82	2.98	hypothetical protein
PA3092	*fadH1*	2.55		2,4-dienoyl-CoA reductase FadH1
PA3104	*xcpP*		-2.83	secretion protein XcpP
PA3112	*accD*		-5.83	acetyl-CoA carboxylase beta subunit
PA3115	*fimV*	2.25		Motility protein FimV
PA3123			4.30	conserved hypothetical protein
PA3126	*ibpA*	2.92		heat-shock protein IbpA
PA3144			3.34	hypothetical protein
PA3147	*wbpJ*	3.05		probable glycosyl transferase WbpJ
PA3148	*wbpI*	5.42	3.49	probable UDP-N-acetylglucosamine 2-epimerase WbpI
PA3149	*wbpH*	3.30		probable glycosyltransferase WbpH
PA3150	*wbpG*	4.08	4.51	LPS biosynthesis protein WbpG
PA3151	*hisF2*	4.23	2.65	imidazoleglycerol-phosphate synthase, cyclase subunit
PA3152	*hisH2*	3.81	4.38	glutamine amidotransferase
PA3153	*wzx*	2.69		O-antigen translocase
PA3154	*wzy*	4.13	2.61	B-band O-antigen polymerase
PA3155	*wbpE*	4.08		probable aminotransferase WbpE
PA3156	*wbpD*	3.08		probable acetyltransferase WbpD
PA3158	*wbpB*	2.68		probable oxidoreductase WpbB
PA3159	*wbpA*	2.45		probable UDP-glucose/GDP-mannose dehydrogenase WbpA
PA3160	*wzz*	3.13		O-antigen chain length regulator
PA3162	*rpsA*		-6.98	30S ribosomal protein S1
PA3166	*pheA*		-3.24	chorismate mutase
PA3173	*yciK*		-2.94	probable short-chain dehydrogenase
PA3190	*gltB*	-7.78	-5.46	probable binding protein component of ABC sugar transporter
PA3202	*yciI*	2.83		conserved hypothetical protein
PA3204			-3.47	probable two-component response regulator

PA3217	*cyaB*		-3.90	CyaB
PA3225			2.71	probable transcriptional regulator
PA3231			6.34	hypothetical protein
PA3237		2.29		hypothetical protein
PA3244	*minD*		-4.15	cell division inhibitor MinD
PA3245	*minE*		-6.45	cell division topological specificity factor MinE
PA3256			2.67	probable oxidoreductase
PA3266	*capB*		-17.32	cold acclimation protein B
PA3273		2.92	3.78	hypothetical protein
PA3274			2.75	hypothetical protein
PA3278		9.75	4.21	hypothetical protein
PA3284		2.21	3.86	hypothetical protein
PA3292			-5.12	hypothetical protein
PA3308	*hepA*		-2.86	RNA helicase HepA
PA3309	*uspK*	3.31	8.31	conserved hypothetical protein
PA3332			-3.72	conserved hypothetical protein
PA3337	*rfaD*	2.45	2.86	ADP-L-glycero-D-mannoheptose 6-epimerase
PA3341			-2.94	probable transcriptional regulator
PA3361	*lecB*	-15.39	-54.35	fucose-binding lectin PA-IIL
PA3385	*amrZ*	-12.48	-3.83	alginate and motility regulator Z
PA3389		2.23		probable ring-cleaving dioxygenase
PA3397	*fpr*		-3.49	ferredoxin--NADP+ reductase
PA3407	*hasAp*	13.89	14.73	heme acquisition protein HasAp
PA3415			2.62	probable dihydrolipoamide acetyltransferase
PA3417			4.84	probable pyruvate dehydrogenase E1 component, alpha subunit
PA3431	*ywbG*	23.70	18.32	conserved hypothetical protein
PA3432		31.75	33.44	hypothetical protein
PA3459	*asnB*		3.46	probable glutamine amidotransferase
PA3461	*yhfE*		4.76	conserved hypothetical protein
PA3472			-3.55	hypothetical protein
PA3476	*rhlI*	-4.14	-4.52	autoinducer synthesis protein RhlI
PA3477	*rhlR*	-9.43	-4.84	transcriptional regulator RhlR
PA3478	*rhlB*		-3.35	rhamnosyltransferase chain B
PA3480	*dcd*		-4.10	probable deoxycytidine triphosphate deaminase
PA3489	*rnfA*		-9.37	conserved hypothetical protein
PA3491	*rnfC*		2.41	probable ferredoxin
PA3496		-3.86	-4.75	hypothetical protein
PA3509		3.08	3.15	probable hydrolase
PA3525	*argG*		-4.17	argininosuccinate synthase
PA3529	*tsaA*		2.99	probable peroxidase
PA3530	*bfd*	-42.81	-15.35	conserved hypothetical protein
PA3531	*bfrB*		-3.61	bacterioferritin
PA3533	*ydhD*	-2.83	-3.78	conserved hypothetical protein
PA3572		2.48	12.69	hypothetical protein
PA3578		2.78	2.81	conserved hypothetical protein
PA3581	*glpF*	2.91		glycerol uptake facilitator protein
PA3584	*glpD*	40.00		glycerol-3-phosphate dehydrogenase
PA3600	*rpl36*	17.30	37.88	conserved hypothetical protein
PA3601	*ykgM*	13.16	10.03	conserved hypothetical protein
PA3613		3.68	4.36	hypothetical protein
PA3621	*fdxA*		-5.47	ferredoxin I
PA3636	*kdsA*		-4.10	2-dehydro-3-deoxyphosphooctonate aldolase

PA3637	*pyrG*		-3.52	CTP synthase
PA3645	*fabZ*		-6.46	(3R)-hydroxymyristoyl-[acyl carrier protein] dehydratase
PA3646	*lpxD*		-6.09	UDP-3-O-[3-hydroxylauroyl] glucosamine N-acyltransferase
PA3648			-3.65	probable outer membrane protein precursor
PA3652	*uppS*		-3.56	undecaprenyl pyrophosphate synthetase
PA3654	*pyrH*		-3.71	uridylate kinase
PA3655	*tsf*		-4.48	elongation factor Ts
PA3656	*rpsB*		-5.15	30S ribosomal protein S2
PA3684		-35.87	-9.39	hypothetical protein
PA3686	*adk*		-6.99	adenylate kinase
PA3687	*ppc*		2.55	phosphoenolpyruvate carboxylase
PA3689	*yhdM*		2.93	probable transcriptional regulator
PA3691			5.57	hypothetical protein
PA3722			-4.80	hypothetical protein
PA3732	*yjfI*	2.97	5.82	conserved hypothetical protein
PA3741			-3.55	hypothetical protein
PA3742	*rplS*		-12.99	50S ribosomal protein L19
PA3743	*trmD*		-5.73	tRNA (guanine-N1)-methyltransferase
PA3744	*rimM*		-3.18	16S rRNA processing protein
PA3745	*rpsP*		-4.23	30S ribosomal protein S16
PA3747			-3.60	conserved hypothetical protein
PA3770	*guaB*		-4.23	inosine-5'-monophosphate dehydrogenase
PA3796			5.72	hypothetical protein
PA3804			-2.99	hypothetical protein
PA3805	*pilF*		-3.97	type 4 fimbrial biogenesis protein PilF
PA3806	*yfgB*		-8.95	conserved hypothetical protein
PA3808	*yfhJ*	-4.80	-3.62	conserved hypothetical protein
PA3812	*iscA*	-3.18	-3.64	probable iron-binding protein IscA
PA3815			-11.22	conserved hypothetical protein
PA3818	*suhB*		-3.44	extragenic suppressor protein SuhB
PA3824	*queA*		-5.40	S-adenosylmethionine:trna ribosyltransferase-isomerase
PA3828	*yjgP*		-9.96	conserved hypothetical protein
PA3841	*exoS*	7.44	16.13	exoenzyme S
PA3842	*orf1*	4.79	3.82	probable chaperone
PA3843		4.19	3.09	hypothetical protein
PA3866			2.27	pyocin protein
PA3880		2.41	2.88	conserved hypothetical protein
PA3905			-3.18	hypothetical protein
PA3906			-3.19	hypothetical protein
PA3908			-4.89	hypothetical protein
PA3916	*moaE*		2.42	molybdopterin converting factor, large subunit
PA3940		-2.72	-6.70	probable DNA binding protein
PA3941			-3.40	hypothetical protein
PA3966			-4.73	hypothetical protein
PA3967			-6.58	hypothetical protein
PA3968	*ymfC*	2.65	2.95	probable pseudouridine synthase
PA3979			-5.30	hypothetical protein
PA3982			-3.89	conserved hypothetical protein
PA3986		-18.81	1.90	hypothetical protein
PA3989	*holA*		-2.99	DNA polymerase III, delta subunit

PA3996	*lis*		-3.55	lipoate synthase
PA4028		2.38		hypothetical protein
PA4031	*ppa*		-3.68	inorganic pyrophosphatase
PA4049		-4.38	-3.22	hypothetical protein
PA4067	*oprG*	3.30		Outer membrane protein OprG precursor
PA4108			2.66	hypothetical protein
PA4117		-6.23	-3.67	probable bacteriophytochrome
PA4129		-3.19	-3.93	hypothetical protein
PA4130			-3.33	probable sulfite or nitrite reductase
PA4133	*ccoN*		-3.90	cytochrome c oxidase subunit (cbb3-type)
PA4134			-9.02	hypothetical protein
PA4139		-5.21	-41.79	hypothetical protein
PA4140			-7.00	hypothetical protein
PA4141			-6.62	hypothetical protein
PA4142			-6.44	probable secretion protein
PA4211	*phzB1*	3.34		probable phenazine biosynthesis protein
PA4217	*phzS*	3.11		flavin-containing monooxygenase
PA4220	*fptB*	-4.07	4.92	hypothetical protein
PA4237	*rplQ*		-7.58	50S ribosomal protein L17
PA4238	*rpoA*		-5.00	DNA-directed RNA polymerase alpha chain
PA4239	*rpsD*		-5.24	30S ribosomal protein S4
PA4240	*rpsK*		-5.02	30S ribosomal protein S11
PA4241	*rpsM*		-8.17	30S ribosomal protein S13
PA4242	*rpmJ*		-4.31	50S ribosomal protein L36
PA4243	*secY*		-3.71	secretion protein SecY
PA4245	*rpmD*		-3.68	50S ribosomal protein L30
PA4246	*rpsE*		-4.03	30S ribosomal protein S5
PA4247	*rplR*		-8.99	50S ribosomal protein L18
PA4248	*rplF*		-3.87	50S ribosomal protein L6
PA4249	*rpsH*		-4.45	30S ribosomal protein S8
PA4250	*rpsN*		-5.22	30S ribosomal protein S14
PA4254	*rpsQ*		-3.75	30S ribosomal protein S17
PA4258	*rplV*		-3.44	50S ribosomal protein L22
PA4261	*rplW*		-6.19	50S ribosomal protein L23
PA4263	*rplC*		-3.32	50S ribosomal protein L3
PA4264	*rpsJ*		-5.72	30S ribosomal protein S10
PA4265	*tufA*	1.93		elongation factor Tu
PA4270	*rpoB*		-3.18	DNA-directed RNA polymerase beta chain
PA4271	*rplL*		-4.95	50S ribosomal protein L7 / L12
PA4273	*rplA*		-5.62	50S ribosomal protein L1
PA4274	*rplK*		-5.04	50S ribosomal protein L11
PA4275	*nusG*		-5.11	transcription antitermination protein NusG
PA4276	*secE*		-5.58	secretion protein SecE
PA4315	*mvaT*	-3.71	-4.55	transcriptional regulator MvaT, P16 subunit
PA4318			-2.87	hypothetical protein
PA4324		-6.10	-4.68	hypothetical protein
PA4325		-3.76	-2.76	hypothetical protein
PA4348		3.58		conserved hypothetical protein
PA4352		7.02	22.99	conserved hypothetical protein
PA4385	*groEL*	3.90	2.73	GroEL protein
PA4386	*groES*	3.19	3.06	GroES protein
PA4405			-4.65	hypothetical protein
PA4406	*lpxC*		-4.85	UDP-3-O-acyl-N-acetylglucosamine deacetylase

PA4424	*yraN*		-3.86	conserved hypothetical protein
PA4426	*yraP*		-5.07	conserved hypothetical protein
PA4427	*sspB*		-5.13	stringent starvation protein B
PA4428	*sspA*		-8.51	stringent starvation protein A
PA4429			-3.70	probable cytochrome c1 precursor
PA4430			-6.26	probable cytochrome b
PA4431			-6.21	probable iron-sulfur protein
PA4432	*rpsI*		-15.10	30S ribosomal protein S9
PA4433	*rplM*		-12.20	50S ribosomal protein L13
PA4440		2.36		hypothetical protein
PA4449	*hisG*		-3.58	ATP-phosphoribosyltransferase
PA4458	*yrbI*		-4.11	conserved hypothetical protein
PA4459	*yrbK*		-4.03	conserved hypothetical protein
PA4460	*yhbN*		-3.29	conserved hypothetical protein
PA4461	*yhbG*		-7.68	probable ATP-binding component of ABC transporter
PA4466			-3.79	probable phosphoryl carrier protein
PA4475			2.92	conserved hypothetical protein
PA4479	*mreD*	3.90		rod shape-determining protein MreD
PA4480	*mreC*		-4.02	rod shape-determining protein MreC
PA4481	*mreB*		-3.10	rod shape-determining protein MreB
PA4494			-4.13	probable two-component sensor
PA4523			5.48	hypothetical protein
PA4528	*pilD*		-2.80	type 4 prepilin peptidase PilD
PA4542	*clpB*	3.48	3.22	ClpB protein
PA4545	*comL*		-4.68	competence protein ComL
PA4563	*rpsT*		-52.87	30S ribosomal protein S20
PA4567	*rpmA*	-2.11	-15.74	50S ribosomal protein L27
PA4568	*rplU*		-7.27	50S ribosomal protein L21
PA4569	*ispB*		-3.26	octaprenyl-diphosphate synthase
PA4575			2.83	hypothetical protein
PA4577		3.99	8.31	hypothetical protein
PA4587	*ccpR*	6.96	2.46	cytochrome c551 peroxidase precursor
PA4602	*glyA3*		-4.33	serine hydroxymethyltransferase
PA4608		-6.99	3.00	hypothetical protein
PA4610		3.61	4.04	hypothetical protein
PA4625			-5.40	hypothetical protein
PA4670	*prs*		-10.97	ribose-phosphate pyrophosphokinase
PA4671	*rplY*		-5.12	probable ribosomal protein L25
PA4672	*pth*		-8.69	peptidyl-tRNA hydrolase
PA4673	*ychF*		-5.27	conserved hypothetical protein
PA4674	*vapI*		3.06	conserved hypothetical protein
PA4678	*rimI*		-4.16	peptide n-acetyltransferase RimI
PA4691			2.88	hypothetical protein
PA4697		2.41		hypothetical protein
PA4699		2.31	2.60	hypothetical protein
PA4728	*folK*		-2.52	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase
PA4741	*rpsO*		-6.58	30S ribosomal protein S15
PA4743	*rbfA*		-7.73	ribosome-binding factor A
PA4746	*yhbC*		-18.43	conserved hypothetical protein
PA4747	*secG*		-4.28	secretion protein SecG
PA4753	*yhbY*		-5.31	conserved hypothetical protein
PA4757	*yeaS*		-5.60	conserved hypothetical protein

PA4761	*dnaK*	5.20	4.56	DnaK protein
PA4762	*grpE*	3.00	2.64	heat shock protein GrpE
PA4765	*omlA*		-8.86	Outer membrane lipoprotein OmlA precursor
PA4768	*smpB*		-3.53	SmpB protein
PA4778	*ybbI*	-17.15	-7.45	probable transcriptional regulator
PA4833			4.21	conserved hypothetical protein
PA4846	*aroQ1*		-4.32	3-dehydroquinate dehydratase
PA4853	*fis*		-12.18	DNA-binding protein Fis
PA4876	*osmE*		3.66	osmotically inducible lipoprotein OsmE
PA4877			4.21	hypothetical protein
PA4881		2.70	3.51	hypothetical protein
PA4932	*rplI*		-5.61	50S ribosomal protein L9
PA4933		2.21		hypothetical protein
PA4934	*rpsR*		-3.92	30S ribosomal protein S18
PA4935	*rpsF*		-3.91	30S ribosomal protein S6
PA4940	*yjeT*	-11.03	-23.64	conserved hypothetical protein
PA4943	*hflX*		-4.30	probable GTP-binding protein
PA4962	*ybcI*		-3.04	conserved hypothetical protein
PA4971	*aspP*		2.71	adenosine diphosphate sugar pyrophosphatase
PA5010	*waaG*	2.47		UDP-glucose:(heptosyl) LPS alpha 1,3-glucosyltransferase WaaG
PA5027		4.30	7.02	hypothetical protein
PA5030	*ynfM*	3.22		probable major facilitator superfamily (MFS) transporter
PA5033			-2.83	hypothetical protein
PA5045	*ponA*		-6.07	penicillin-binding protein 1A
PA5046			-4.26	malic enzyme
PA5049	*rpmE*		-12.29	50S ribosomal protein L31
PA5051	*argS*	3.45		arginyl-tRNA synthetase
PA5054	*hslU*	2.67		heat shock protein HslU
PA5055			-2.96	hypothetical protein
PA5105	*hutC*	2.87	2.72	histidine utilization repressor HutC
PA5106		8.55	8.78	conserved hypothetical protein
PA5117	*typA*		-4.40	regulatory protein TypA
PA5118	*thiI*		-3.71	thiazole biosynthesis protein ThiI
PA5129	*grx*		-4.06	glutaredoxin
PA5130	*yibN*		-2.48	conserved hypothetical protein
PA5139			-3.03	hypothetical protein
PA5163	*rmlA*	-5.53	-3.24	glucose-1-phosphate thymidylyltransferase
PA5170	*arcD*		14.77	arginine/ornithine antiporter
PA5171	*arcA*	6.64	15.85	arginine deiminase
PA5172	*arcB*	4.39	16.37	ornithine carbamoyltransferase, catabolic
PA5173	*arcC*		8.22	carbamate kinase
PA5202		2.28		hypothetical protein
PA5212			3.54	hypothetical protein
PA5232	*yhiI*	2.75	4.38	conserved hypothetical protein
PA5239	*rho*		-12.11	transcription termination factor Rho
PA5261	*algR*		3.66	alginate biosynthesis regulatory protein AlgR
PA5263	*argH*		-2.46	argininosuccinate lyase
PA5271			2.53	hypothetical protein
PA5276	*lppL*		-3.71	Lipopeptide LppL precursor
PA5300	*cycB*		-4.18	cytochrome c5
PA5301	*ycjC ;*	-4.88	-3.01	probable transcriptional regulator

PA5315	*rpmG*	1.15	-12.03	50S ribosomal protein L33
PA5316	*rpmB*	-2.32	-17.01	50S ribosomal protein L28
PA5338	*spoT*		-5.70	guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase
PA5348		-32.19	-3.67	probable DNA-binding protein
PA5355	*glcD*	2.12		glycolate oxidase subunit GlcD
PA5366	*pstB*		-4.19	ATP-binding component of ABC phosphate transporter
PA5367	*pstA*		-3.52	membrane protein component of ABC phosphate transporter
PA5373	*betB*	4.91	3.92	betaine aldehyde dehydrogenase
PA5374	*betI*	9.91	2.53	transcriptional regulator BetI
PA5388		2.75	2.65	hypothetical protein
PA5408			5.51	hypothetical protein
PA5409			4.87	hypothetical protein
PA5427	*adhA*	15.46	17.95	alcohol dehydrogenase
PA5435	*oadA*		-3.05	probable transcarboxylase subunit
PA5461		-11.46	-7.21	hypothetical protein
PA5475		5.59	6.98	hypothetical protein
PA5479	*gltP*		-5.20	proton-glutamate symporter
PA5490	*cc4*		-8.03	cytochrome c4 precursor
PA5491		-4.38	-19.91	probable cytochrome
PA5494			6.36	hypothetical protein
PA5504			-2.98	probable permease of ABC transporter
PA5505			-5.24	probable TonB-dependent receptor
PA5546			2.46	conserved hypothetical protein
PA5560	*atpB*		-7.49	ATP synthase A chain
PA5561	*atpI*	-3.26	-5.89	ATP synthase protein I
PA5563	*soj*	-3.29	-7.82	chromosome partitioning protein Soj
PA5568	*yidC*		-7.11	conserved hypothetical protein
PA5569	*rnpA*		-8.68	ribonuclease P protein component
PA5570	*rpmH*		-3.84	50S ribosomal protein L34
5S rRNA		-3.10	-3.23	5S ribosomal RNA
AF035937cds3	*wbpV*		-4.65	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF035937cds6	*wbpO*		-3.82	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF043558cds			-3.09	Pseudomonas aeruginosa beta-lactamase OXA-13-1 gene, complete cds.
AF191564cds4	*sul1*		-3.29	metallo beta-lactamase
AF241171cds1		4.01	4.98	No significant similarity
AF241171cds39		2.41	2.79	first half of finger motif
J05162cds			-4.31	*P .aeruginosa* carbenicillinase gene, complete cds.
L81176cds6		-4.48	-5.03	*P. aeruginosa* flagellin (fliC) gene, partial cds
M21651cds		4.18	3.18	*P. aeruginosa* K122-4 type IV pilin precursor
M57501cds	*flaA*	-4.53	-3.44	Pseudomonas aeruginosa flagellin (flaA) gene
Pae_orfB		-5.20	-4.44	putative acyl carrier protein - fatty acid biosynthesis, from glycosylation island genes in strain PAK
Pae_orfC		-7.35	-3.99	putative 3-oxoacyl (acyl carrier protein) synthase - fatty acid biosnthesis, from glycosylation island genes in strain PAK
Pae_tRNA_Ala			-13.35	tRNA_Alanine
Pae_tRNA_Arg		-7.84	-3.26	tRNA_Arginine

Pae_tRNA_Asn	-3.14	-18.45	tRNA_Asparagine
Pae_tRNA_Cys	-9.16	-8.16	tRNA_Cysteine
Pae_tRNA_Gln		-13.88	tRNA_Glutamine
Pae_tRNA_Gly	-3.52	-20.35	tRNA_Glycine
Pae_tRNA_His		-6.85	tRNA_Histidine
Pae_tRNA_Leu	-12.87	-35.11	tRNA_Leucine
Pae_tRNA_Lys		-4.53	tRNA_Lysine
Pae_tRNA_Phe		-9.47	tRNA_Phenylalanine
Pae_tRNA_Pro		-3.21	tRNA_Proline
Pae_tRNA_Trp		-5.77	tRNA_Tryptophan
Pae_tRNA_Tyr		-3.63	tRNA_Tyrosine
Pae_tRNA_Val	-5.31	-11.82	tRNA_Valine

Table 8-3: Differentially regulated genes (pfp < 0.05) in lettuce leaf infection in comparison with planktonic and biofilm growth controls.

PA Number	Gene	Fold change compared to Planktonic	Biofilm	Product Name
PA0007		-2.86		hypothetical protein
PA0011		2.25		probable 2-OH-lauroyltransferase
PA0035	*trpA*	2.22	2.69	tryptophan synthase alpha chain
PA0038		-3.67		hypothetical protein
PA0050			-4.04	hypothetical protein
PA0052			7.28	hypothetical protein
PA0059	*osmC*		3.04	osmotically inducible protein OsmC
PA0085		3.49		conserved hypothetical protein
PA0089			-2.51	hypothetical protein
PA0102		3.04	4.93	probable carbonic anhydrase
PA0105	*coxB*	-4.13	3.83	cytochrome c oxidase, subunit II
PA0106	*coxA*	-10.50		cytochrome c oxidase, subunit I
PA0107		-8.70	2.79	conserved hypothetical protein
PA0108	*coIII*	-22.22		cytochrome c oxidase, subunit III
PA0109			3.97	hypothetical protein
PA0110		-5.95		hypothetical protein
PA0111		-8.81		hypothetical protein
PA0112		-2.61		hypothetical protein
PA0113		-6.29		probable cytochrome c oxidase assembly factor
PA0122		-5.99		conserved hypothetical protein
PA0128	*phnA*		-2.77	conserved hypothetical protein
PA0140	*ahpF*		-3.16	alkyl hydroperoxide reductase subunit F
PA0156	*triA*	-3.76		Resistance-Nodulation-Cell Division (RND) triclosan efflux membrane fusion protein, TriA
PA0157	*triB*	-2.99		Resistance-Nodulation-Cell Division (RND) triclosan efflux membrane fusion protein, TriB
PA0160			-2.64	hypothetical protein
PA0161			-2.96	hypothetical protein
PA0172			-2.59	hypothetical protein
PA0173		-3.20		probable methylesterase
PA0175	*cheR2*	-5.18		probable chemotaxis protein methyltransferase
PA0176	*aer2*	-8.64		aerotaxis transducer Aer2
PA0177		-8.41		probable purine-binding chemotaxis protein
PA0178		-5.33		probable two-component sensor
PA0179		-5.57	3.49	probable two-component response regulator
PA0180		-4.13		probable chemotaxis transducer
PA0200			9.62	hypothetical protein
PA0201		2.84	3.59	hypothetical protein
PA0226		2.89		probable CoA transferase, subunit A
PA0249		-3.69		probable acetyltransferase
PA0250			2.41	conserved hypothetical protein
PA0256		-3.53		hypothetical protein
PA0257			-3.26	hypothetical protein
PA0258			-3.89	hypothetical protein
PA0261			-2.93	hypothetical protein
PA0263	*hcpC*	3.41	-24.45	secreted protein Hcp
PA0276		10.09	6.49	hypothetical protein

PA0280	*cysA*	6.63	6.75	sulfate transport protein CysA
PA0281	*cysW*	6.80	5.95	sulfate transport protein CysW
PA0282	*cysT*	3.37	2.72	sulfate transport protein CysT
PA0283	*sbp*	8.56	8.41	sulfate-binding protein precursor
PA0284		5.99	7.22	hypothetical protein
PA0286	*desA*	11.07	8.54	delta-9 fatty acid desaturase, DesA
PA0291	*oprE*		-3.97	Anaerobically-induced outer membrane porin OprE precursor
PA0297	*spuA*	3.57		probable glutamine amidotransferase
PA0298	*spuB*	2.94	2.46	probable glutamine synthetase
PA0312			4.83	conserved hypothetical protein
PA0315		-3.64		hypothetical protein
PA0329		-5.75		conserved hypothetical protein
PA0332			4.05	hypothetical protein
PA0355	*pfpI*	-3.90		protease PfpI
PA0359			-2.78	hypothetical protein
PA0363	*coaD*		-2.80	phosphopantetheine adenylyltransferase
PA0365		-5.55		hypothetical protein
PA0366		-3.30		probable aldehyde dehydrogenase
PA0377		2.75		hypothetical protein
PA0385		3.98		hypothetical protein
PA0386	*yggW*	2.61		probable oxidase
PA0395	*pilT*	-2.28		twitching motility protein PilT
PA0408	*pilG*		-4.53	twitching motility protein PilG
PA0409	*pilH*		-5.16	twitching motility protein PilH
PA0410	*pilI*		-2.77	twitching motility protein PilI
PA0411	*pilJ*		-2.98	twitching motility protein PilJ
PA0424	*mexR*		-2.95	multidrug resistance operon repressor MexR
PA0432	*sahH*	-3.09	-3.08	S-adenosyl-L-homocysteine hydrolase
PA0436		2.81		probable transcriptional regulator
PA0439		2.55	3.14	probable oxidoreductase
PA0441	*dht*	4.15	5.15	dihydropyrimidinase
PA0444		2.87		N-carbamoyl-beta-alanine amidohydrolase
PA0447	*gcdH*		-7.56	glutaryl-CoA dehydrogenase
PA0451		4.11	10.32	conserved hypothetical protein
PA0452	*slp*		3.65	probable stomatin-like protein
PA0456		22.78	3.57	probable cold-shock protein
PA0459	*clpC*	-3.49		probable ClpA/B protease ATP binding subunit
PA0460		-3.27		hypothetical protein
PA0469			3.56	hypothetical protein
PA0472	*fiuI*	2.43	9.38	probable sigma-70 factor, ECF subfamily
PA0484			4.65	conserved hypothetical protein
PA0485	*rarD*	2.90		conserved hypothetical protein
PA0490			3.15	hypothetical protein
PA0505		2.54	14.22	hypothetical protein
PA0506			-3.09	probable acyl-CoA dehydrogenase
PA0520	*nirQ*		2.64	regulatory protein NirQ
PA0536		-2.39		hypothetical protein
PA0538	*dsbB*	2.55	2.48	disulfide bond formation protein
PA0540		-2.79		hypothetical protein
PA0541			-3.39	hypothetical protein
PA0546	*metK*		-2.57	methionine adenosyltransferase
PA0553		-4.86		hypothetical protein

PA0555	*fda*	-3.35		fructose-1,6-bisphosphate aldolase
PA0563		2.61		conserved hypothetical protein
PA0566		2.51		hypothetical protein
PA0567	*yqaE*	-4.84		conserved hypothetical protein
PA0576	*rpoD*		-2.67	sigma factor RpoD
PA0578		4.53	-7.98	conserved hypothetical protein
PA0579	*rpsU*	7.62		30S ribosomal protein S21
PA0580	*gcp*	2.67		O-sialoglycoprotein endopeptidase
PA0581	*ygiH*		-2.56	conserved hypothetical protein
PA0586	*ycgB*	-5.65		conserved hypothetical protein
PA0588	*yeaG*	-3.28		conserved hypothetical protein
PA0589	*glpE*		-3.79	conserved hypothetical protein
PA0595	*ostA*		-3.50	organic solvent tolerance protein OstA precursor
PA0608	*gph*	3.23		probable phosphoglycolate phosphatase
PA0610	*prtN*	-2.61		transcriptional regulator PrtN
PA0612	*ptrB*	-4.30		repressor, PtrB
PA0614		-2.72		hypothetical protein
PA0616		-2.84		hypothetical protein
PA0619		-2.82		probable bacteriophage protein
PA0623		-2.78		probable bacteriophage protein
PA0624		-5.33		hypothetical protein
PA0631		-3.17		hypothetical protein
PA0635		-3.29		hypothetical protein
PA0636		-2.70		hypothetical protein
PA0648		-2.81		hypothetical protein
PA0652	*vfr*		-3.42	transcriptional regulator Vfr
PA0654	*speD*		-3.84	S-adenosylmethionine decarboxylase proenzyme
PA0655			-2.32	hypothetical protein
PA0656	*ycfF*		3.06	probable HIT family protein
PA0665	*yadR*	2.98		conserved hypothetical protein
PA0667	*yebA*		-2.75	conserved hypothetical protein
PA0676		3.18		probable transmembrane sensor
PA0713			-2.77	hypothetical protein
PA0730		3.19		probable transferase
PA0734		24.45	12.76	hypothetical protein
PA0745		-4.34		probable enoyl-CoA hydratase/isomerase
PA0746		-2.76		probable acyl-CoA dehydrogenase
PA0747		-2.96		probable aldehyde dehydrogenase
PA0762	*algU*	-2.90		sigma factor AlgU
PA0766	*mucD*		-3.24	serine protease MucD precursor
PA0767	*lepA*		-5.09	GTP-binding protein LepA
PA0768	*lepB*		-2.96	signal peptidase I
PA0776		-2.66		hypothetical protein
PA0801			2.47	hypothetical protein
PA0802		1.92	2.83	hypothetical protein
PA0805		3.39		hypothetical protein
PA0810			2.29	probable haloacid dehalogenase
PA0814		3.90	4.25	conserved hypothetical protein
PA0815		6.47	11.42	probable transcriptional regulator
PA0817		2.60	2.68	probable ring-cleaving dioxygenase
PA0837	*slyD*	2.62	3.17	peptidyl-prolyl cis-trans isomerase SlyD
PA0838	*btuE*		2.62	probable glutathione peroxidase
PA0851		2.67	2.74	hypothetical protein

PA0856		2.75		hypothetical protein
PA0859			2.74	hypothetical protein
PA0862			2.97	hypothetical protein
PA0865	*hpd*	-7.16		4-hydroxyphenylpyruvate dioxygenase
PA0866	*aroP2*	-3.19		aromatic amino acid transport protein AroP2
PA0870	*phhC*	-5.86		aromatic amino acid aminotransferase
PA0871	*phhB*	-6.65		pterin-4-alpha-carbinolamine dehydratase
PA0872	*phhA*	-3.32		phenylalanine-4-hydroxylase
PA0892	*aotP*	-2.36		arginine/ornithine transport protein AotP
PA0900		-3.11		hypothetical protein
PA0909			2.63	hypothetical protein
PA0915	*yehS*		-2.66	conserved hypothetical protein
PA0921		-6.15		hypothetical protein
PA0929	*pirR*	3.14	6.63	two-component response regulator
PA0936	*lpxO2*	3.56		lipopolysaccharide biosynthetic protein LpxO2
PA0942			2.37	probable transcriptional regulator
PA0944	*purN*		-3.94	phosphoribosylaminoimidazole synthetase
PA0952			-4.89	hypothetical protein
PA0954			2.49	probable acylphosphatase
PA0955			-3.98	hypothetical protein
PA0959		-3.38		hypothetical protein
PA0961			-2.87	probable cold-shock protein
PA0962		-2.81		probable dna-binding stress protein
PA0964	*yebC*		-3.06	conserved hypothetical protein
PA0969	*tolQ*		-4.03	TolQ protein
PA0974			-3.02	conserved hypothetical protein
PA0981			-3.44	hypothetical protein
PA0988			2.95	hypothetical protein
PA0995	*ogt*	2.50	3.04	methylated-DNA--protein-cysteine methyltransferase
PA0998	*pqsC*		-2.93	Homologous to beta-keto-acyl-acyl-carrier protein synthase
PA1001	*phnA*		-3.19	anthranilate synthase component I
PA1011			-2.71	hypothetical protein
PA1027	*pcd*	-2.96		probable aldehyde dehydrogenase
PA1029			2.69	hypothetical protein
PA1034			-2.77	hypothetical protein
PA1035		-3.62		hypothetical protein
PA1041		-6.80	2.52	probable outer membrane protein precursor
PA1048		-4.22		probable outer membrane protein precursor
PA1060		3.14	4.04	hypothetical protein
PA1075			3.24	hypothetical protein
PA1080	*flgE*	-2.61	-3.47	flagellar hook protein FlgE
PA1097	*fleQ*	-4.45		transcriptional regulator FleQ
PA1118			4.79	hypothetical protein
PA1121		-3.61		conserved hypothetical protein
PA1123			-25.40	hypothetical protein
PA1132			-3.24	hypothetical protein
PA1151	*imm2*	-3.66		pyocin S2 immunity protein
PA1159		5.35	3.24	probable cold-shock protein
PA1160		2.84	3.11	hypothetical protein
PA1168		-5.54	-62.11	hypothetical protein
PA1174	*napA*	-4.68		periplasmic nitrate reductase protein NapA
PA1175	*napD*	-2.78		NapD protein of periplasmic nitrate reductase

PA1176	*napF*	-3.83	3.26	ferredoxin protein NapF
PA1177	*napE*		26.32	periplasmic nitrate reductase protein NapE
PA1178	*oprH*	-56.76		PhoP/Q and low Mg2+ inducible outer membrane protein H1 precursor
PA1179	*phoP*	-11.64		two-component response regulator PhoP
PA1180	*phoQ*	-6.58		two-component sensor PhoQ
PA1183	*dctA*	10.38	-2.67	C4-dicarboxylate transport protein
PA1190	*yohC*	5.41	35.09	conserved hypothetical protein
PA1192	*ydaO*	2.84		conserved hypothetical protein
PA1193			-2.77	hypothetical protein
PA1198			-3.28	conserved hypothetical protein
PA1199			-4.45	probable lipoprotein
PA1202	*ycaC*	-4.67		probable hydrolase
PA1245	*aprX*	-3.41		hypothetical protein
PA1246	*aprD*	-3.43		alkaline protease secretion protein AprD
PA1247	*aprE*	-2.83		alkaline protease secretion protein AprE
PA1249	*aprA*	-3.30		alkaline metalloproteinase precursor
PA1283		-3.19		probable transcriptional regulator
PA1287			2.53	probable glutathione peroxidase
PA1289		-6.34		hypothetical protein
PA1296		2.50		probable 2-hydroxyacid dehydrogenase
PA1300		-1.81		probable sigma-70 factor, ECF subfamily
PA1305		3.07		hypothetical protein
PA1306		2.81		probable HIT family protein
PA1323			2.41	hypothetical protein
PA1327		-2.68		probable protease
PA1340		-4.20	-2.87	probable permease of ABC transporter
PA1342		-2.99		probable binding protein component of ABC transporter
PA1348		-9.99		hypothetical protein
PA1377	*yhhY*		4.05	conserved hypothetical protein
PA1404			10.60	hypothetical protein
PA1414			3.35	hypothetical protein
PA1430	*lasR*	-2.36		transcriptional regulator LasR
PA1432	*lasI*		3.41	autoinducer synthesis protein LasI
PA1440			2.74	hypothetical protein
PA1457	*cheZ*	-2.28		chemotaxis protein CheZ
PA1464	*cheW*		5.12	probable purine-binding chemotaxis protein
PA1476	*ccmB*		-2.34	heme exporter protein CcmB
PA1478	*ccmD*		-3.14	hypothetical protein
PA1480	*ccmF*		-2.90	cytochrome C-type biogenesis protein CcmF
PA1482	*ccmH*		-5.76	cytochrome C-type biogenesis protein CcmH
PA1505	*moaA2*	2.95	5.79	molybdopterin biosynthetic protein A2
PA1511			-3.26	conserved hypothetical protein
PA1517		2.60	4.71	conserved hypothetical protein
PA1533		-3.01	-2.65	conserved hypothetical protein
PA1540			-2.92	conserved hypothetical protein
PA1544	*anr*	2.32		transcriptional regulator Anr
PA1545			3.95	hypothetical protein
PA1550			-3.95	hypothetical protein
PA1551	*fixG*		-4.05	probable ferredoxin
PA1552			-2.71	probable cytochrome c
PA1553	*fixO*	-3.45	-5.69	probable cytochrome c oxidase subunit

PA1555	*fixP*		-19.20	probable cytochrome c
PA1556	*ccoO*		-17.85	probable cytochrome c oxidase subunit
PA1557	*ccoN*		-7.13	probable cytochrome oxidase subunit (cbb3-type)
PA1580	*gltA*		-4.84	citrate synthase
PA1581	*sdhC*	3.63		succinate dehydrogenase (C subunit)
PA1582	*sdhD*	2.38		succinate dehydrogenase (D subunit)
PA1584	*sdhB*		-2.74	succinate dehydrogenase (B subunit)
PA1586	*sucB*	-3.41	-3.06	dihydrolipoamide succinyltransferase (E2 subunit)
PA1588	*sucC*		-2.67	succinyl-CoA synthetase beta chain
PA1596	*htpG*		-4.25	heat shock protein HtpG
PA1610	*fabA*	2.54		beta-hydroxydecanoyl-ACP dehydrase
PA1617		-3.72		probable AMP-binding enzyme
PA1626		2.27	2.62	probable major facilitator superfamily (MFS) transporter
PA1630		3.09	3.55	probable transcriptional regulator
PA1632	*kdpF*	2.34		KdpF protein
PA1641		-5.26		hypothetical protein
PA1645		2.64	4.28	hypothetical protein
PA1651		6.74	5.35	probable transporter
PA1656			-15.39	hypothetical protein
PA1657			-5.24	conserved hypothetical protein
PA1658			-10.97	conserved hypothetical protein
PA1659			-11.02	hypothetical protein
PA1660			-11.14	hypothetical protein
PA1661			-7.27	hypothetical protein
PA1668			-3.49	hypothetical protein
PA1669			-2.94	hypothetical protein
PA1676			4.81	hypothetical protein
PA1677		2.44	10.46	conserved hypothetical protein
PA1701	*pcr3*		-4.44	conserved hypothetical protein in type III secretion
PA1710	*exsC*	-4.30		ExsC, exoenzyme S synthesis protein C precursor.
PA1713	*exsA*		-8.18	transcriptional regulator ExsA
PA1714	*exsD*		-3.22	ExsD
PA1728			27.25	hypothetical protein
PA1729			3.24	conserved hypothetical protein
PA1730			2.83	conserved hypothetical protein
PA1733			2.97	conserved hypothetical protein
PA1741		2.27		hypothetical protein
PA1742		2.45	2.60	probable amidotransferase
PA1751			2.87	hypothetical protein
PA1752			2.43	hypothetical protein
PA1754	*cysB*	-2.77		transcriptional regulator CysB
PA1762		-2.61		hypothetical protein
PA1767			-3.31	hypothetical protein
PA1768			-3.48	hypothetical protein
PA1775	*cmpX*	3.73		conserved cytoplasmic membrane protein, CmpX protein
PA1778	*cobA*	4.03	4.24	uroporphyrin-III C-methyltransferase
PA1779	*nasC*	3.59	3.51	assimilatory nitrate reductase
PA1780	*nirD*	7.32	5.99	assimilatory nitrite reductase small subunit
PA1781	*nirB*	3.86	3.91	assimilatory nitrite reductase large subunit
PA1782		3.05		probable serine/threonine-protein kinase
PA1783	*nasA*	4.05	4.02	nitrate transporter

PA1784		-4.19		hypothetical protein
PA1800	*tig*	2.33		trigger factor
PA1804	*hupB*	-2.89		DNA-binding protein HU
PA1805	*ppiD*		-3.58	peptidyl-prolyl cis-trans isomerase D
PA1812	*mltD*		-7.41	membrane-bound lytic murein transglycosylase D precursor
PA1814			2.32	hypothetical protein
PA1817			3.25	hypothetical protein
PA1819	*yjdE*	-2.93		probable amino acid permease
PA1830			-4.87	hypothetical protein
PA1833	*yhfP*		2.71	probable oxidoreductase
PA1837		3.45	3.58	hypothetical protein
PA1838	*cysI*	3.46	2.85	sulfite reductase
PA1847	*yhgI*	3.95		conserved hypothetical protein
PA1852		3.94		hypothetical protein
PA1856		2.90	3.23	probable cytochrome oxidase subunit
PA1872			3.50	hypothetical protein
PA1874			3.18	hypothetical protein
PA1875	*opmL*	-3.10	5.98	probable outer membrane protein precursor
PA1876		-3.01		probable ATP-binding/permease fusion ABC transporter
PA1887		-9.80		hypothetical protein
PA1888		-5.64		hypothetical protein
PA1901	*phzC2*	-2.21		phenazine biosynthesis protein PhzC
PA1912		2.59	3.30	probable sigma-70 factor, ECF subfamily
PA1914	*hvn*	-3.74		conserved hypothetical protein
PA1927	*metE*	18.66	17.21	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase
PA1931		-2.69	2.67	probable ferredoxin
PA1944		-3.00		hypothetical protein
PA1946	*rbsB*	2.65		binding protein component precursor of ABC ribose transporter
PA1950	*rbsK*		4.03	ribokinase
PA1963		4.28	6.69	hypothetical protein
PA1970		4.44	4.35	hypothetical protein
PA1984	*exaC1*	10.91	3.62	probable aldehyde dehydrogenase
PA1985	*pqqA*		4.84	pyrroloquinoline quinone biosynthesis protein A
PA1998		2.63	2.59	probable transcriptional regulator
PA2006		-2.97		probable major facilitator superfamily (MFS) transporter
PA2007	*maiA*	-6.68	2.58	maleylacetoacetate isomerase
PA2008	*fahA*	-4.87		fumarylacetoacetase
PA2009	*hmgA*	-3.74		homogentisate 1,2-dioxygenase
PA2016	*liuR*	3.33		regulator of liu genes
PA2019	*amrA*	3.85		Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein precursor
PA2020	*amrR*		3.57	probable transcriptional regulator
PA2024			12.06	probable ring-cleaving dioxygenase
PA2025	*gor*		2.41	glutathione reductase
PA2026	*yfeH*		2.44	conserved hypothetical protein
PA2027		-39.54		hypothetical protein
PA2030		8.66	38.76	hypothetical protein
PA2031		30.40	82.64	hypothetical protein
PA2034		-2.97		hypothetical protein

PA2045	*yidD*	3.35	2.66	conserved hypothetical protein
PA2071	*fusA2*	-4.49		elongation factor G
PA2092		2.66	2.42	probable major facilitator superfamily (MFS) transporter
PA2112			-2.30	conserved hypothetical protein
PA2114			-3.11	probable major facilitator superfamily (MFS) transporter
PA2116			-3.02	conserved hypothetical protein
PA2134			3.76	hypothetical protein
PA2143			9.20	hypothetical protein
PA2146	*yciG*		54.64	conserved hypothetical protein
PA2149		-2.55		hypothetical protein
PA2152			2.61	probable trehalose synthase
PA2159		-3.30	2.51	conserved hypothetical protein
PA2166		-2.40	8.88	hypothetical protein
PA2169			5.46	hypothetical protein
PA2170			9.66	hypothetical protein
PA2172			2.88	hypothetical protein
PA2173			2.64	hypothetical protein
PA2174			4.48	hypothetical protein
PA2190			7.17	conserved hypothetical protein
PA2210		2.20		probable major facilitator superfamily (MFS) transporter
PA2229	*yiiM*	2.98	3.17	conserved hypothetical protein
PA2231	*pslA*		-3.38	PslA
PA2233	*pslC*	-4.21	-2.91	probable glycosyl transferase
PA2234	*pslD*	-3.24	-2.82	PslD
PA2235	*pslE*	-2.47		hypothetical protein
PA2237	*pslG*	-2.43		probable glycosyl hydrolase
PA2240	*pslJ*		-2.44	hypothetical protein
PA2247	*bkdA1*	-15.61	-2.50	2-oxoisovalerate dehydrogenase (alpha subunit)
PA2248	*bkdA2*	-9.80		2-oxoisovalerate dehydrogenase (beta subunit)
PA2249	*bkdB*	-10.17		branched-chain alpha-keto acid dehydrogenase (lipoamide component)
PA2250	*lpdV*	-12.16		lipoamide dehydrogenase-Val
PA2259	*ptxS*	8.63	17.39	transcriptional regulator PtxS
PA2260	*kguE*	17.27	16.81	hypothetical protein
PA2261	*kguK*	3.38	3.46	probable 2-ketogluconate kinase
PA2263	*kguD*	3.89	4.78	probable 2-hydroxyacid dehydrogenase
PA2306		12.14	19.61	conserved hypothetical protein
PA2321	*gntV*	2.46		gluconokinase
PA2322	*gntU*	3.06	2.93	gluconate permease
PA2364			3.35	hypothetical protein
PA2372		-2.79	-3.06	hypothetical protein
PA2375			9.18	hypothetical protein
PA2380		10.80	7.86	hypothetical protein
PA2381		-18.11	-5.12	hypothetical protein
PA2383			2.53	probable transcriptional regulator
PA2384			6.28	hypothetical protein
PA2404			2.42	hypothetical protein
PA2405		3.27	3.77	hypothetical protein
PA2409		2.18		probable permease of ABC transporter
PA2411		2.52	6.43	probable thioesterase

PA2412		4.32	9.87	conserved hypothetical protein
PA2414		-4.26		L-sorbosone dehydrogenase
PA2422		-3.46		hypothetical protein
PA2426	*pvdS*	1.79	3.45	sigma factor PvdS
PA2433		-6.31	2.79	hypothetical protein
PA2434		-2.46		hypothetical protein
PA2456			-4.10	hypothetical protein
PA2457			-2.33	hypothetical protein
PA2459			-4.64	hypothetical protein
PA2467	*foxR*		3.84	Anti-sigma factor FoxR
PA2468	*foxI*		2.39	ECF sigma factor FoxI
PA2484			3.87	conserved hypothetical protein
PA2491			5.89	probable oxidoreductase
PA2501		-6.80		hypothetical protein
PA2504		-4.41		hypothetical protein
PA2511			2.88	probable transcriptional regulator
PA2519	*xylS*	3.19	3.36	transcriptional regulator XylS
PA2532	*tpx*		2.79	thiol peroxidase
PA2560		2.63	4.57	hypothetical protein
PA2562		-2.54	2.86	hypothetical protein
PA2564	*tam*	-8.50		hypothetical protein
PA2565		-8.28	2.95	hypothetical protein
PA2566		-13.64		conserved hypothetical protein
PA2568			2.30	hypothetical protein
PA2570	*lecA*	3.18	5.66	LecA
PA2571		-6.56		probable two-component sensor
PA2572		-2.51		probable two-component response regulator
PA2573			5.25	probable chemotaxis transducer
PA2577		-2.78		probable transcriptional regulator
PA2581		-2.60		hypothetical protein
PA2582			2.39	hypothetical protein
PA2584	*pgsA*	5.09		CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase
PA2587	*pqsH*	-3.45		probable FAD-dependent monooxygenase
PA2593			-6.05	hypothetical protein
PA2605	*yheN*		2.82	conserved hypothetical protein
PA2606	*yheM*		3.26	conserved hypothetical protein
PA2607			2.67	conserved hypothetical protein
PA2612	*serS*		-2.35	seryl-tRNA synthetase
PA2615	*ftsK*		2.33	cell division protein FtsK
PA2619	*infA*	3.28	-3.60	initiation factor
PA2620	*clpA*		2.60	ATP-binding protease component ClpA
PA2621			2.94	conserved hypothetical protein
PA2622	*cspD*		2.72	cold-shock protein CspD
PA2624	*idh*		-2.86	isocitrate dehydrogenase
PA2629	*purB*	3.23		adenylosuccinate lyase
PA2639	*nuoD*		-2.58	NADH dehydrogenase I chain C,D
PA2640	*nuoE*	-2.82	-2.89	NADH dehydrogenase I chain E
PA2644	*nuoI*	-3.02	-3.29	NADH Dehydrogenase I chain I
PA2645	*nuoJ*	-2.39	-2.57	NADH dehydrogenase I chain J
PA2646	*nuoK*	-2.83	-2.67	NADH dehydrogenase I chain K
PA2647	*nuoL*	-2.60	-2.51	NADH dehydrogenase I chain L
PA2662		13.53	13.14	conserved hypothetical protein

PA2663		14.08	14.22	hypothetical protein
PA2664	*fhp*	42.02	42.92	flavohemoprotein
PA2666	*ptpS*	3.84		probable 6-pyruvoyl tetrahydrobiopterin synthase
PA2667			-3.60	conserved hypothetical protein
PA2705		-2.48		hypothetical protein
PA2709	*cysK*		3.19	cysteine synthase A
PA2710			2.98	hypothetical protein
PA2718			2.44	probable transcriptional regulator
PA2737		-3.64		conserved hypothetical protein
PA2738	*himA*	-2.68		integration host factor, alpha subunit
PA2741	*rplT*	2.70		50S ribosomal protein L20
PA2743	*infC*	4.16		translation initiation factor IF-3
PA2746		6.73	101.01	hypothetical protein
PA2747			3.48	hypothetical protein
PA2754		-3.92		conserved hypothetical protein
PA2756			4.07	hypothetical protein
PA2759		5.63	8.97	hypothetical protein
PA2762		-2.61		hypothetical protein
PA2769			-4.48	hypothetical protein
PA2771		-3.46		conserved hypothetical protein
PA2779			2.94	hypothetical protein
PA2786		4.70	4.89	hypothetical protein
PA2799			5.13	hypothetical protein
PA2827	*yeaA*		3.55	conserved hypothetical protein
PA2840	*deaD*	4.09		probable ATP-dependent RNA helicase
PA2841		-2.43		probable enoyl-CoA hydratase/isomerase
PA2844			2.29	conserved hypothetical protein
PA2847		312.50	312.50	conserved hypothetical protein
PA2849		-6.22		probable transcriptional regulator
PA2851	*efp*	2.65		translation elongation factor P
PA2897			5.85	probable transcriptional regulator
PA2899		-2.21		probable transcriptional regulator
PA2906		2.76		probable oxidoreductase
PA2918			2.92	probable short-chain dehydrogenase
PA2937			8.26	hypothetical protein
PA2939	*pepB*	-4.15		probable aminopeptidase
PA2946		2.27	2.68	hypothetical protein
PA2950		2.44		hypothetical protein
PA2953			-3.36	electron transfer flavoprotein-ubiquinone oxidoreductase
PA2957		2.58		probable transcriptional regulator
PA2960	*pilZ*		-2.85	type 4 fimbrial biogenesis protein PilZ
PA2966	*acpP*	4.19		acyl carrier protein
PA2970	*rpmF*	2.87		50S ribosomal protein L32
PA2971	*yceD*	3.10		conserved hypothetical protein
PA2985		5.00	3.22	hypothetical protein
PA3006	*psrA*		-2.83	transcriptional regulator PsrA
PA3009		9.73		hypothetical protein
PA3011	*topA*	-3.03	-2.70	DNA topoisomerase I
PA3013	*foaB*		-5.59	fatty-acid oxidation complex beta-subunit
PA3014	*faoA*		-2.55	fatty-acid oxidation complex alpha-subunit
PA3017			10.72	conserved hypothetical protein
PA3031			-2.56	hypothetical protein

PA3032	*snr1*	-3.18		cytochrome c Snr1
PA3033		2.32		hypothetical protein
PA3034		2.53	2.42	probable transcriptional regulator
PA3040	*yqjD*	-2.65	2.73	conserved hypothetical protein
PA3041	*yqjE*		2.33	hypothetical protein
PA3042		-2.90		hypothetical protein
PA3049	*rmf*		4.75	ribosome modulation factor
PA3050	*pyrD*	2.72		dihydroorotate dehydrogenase
PA3057			-3.79	hypothetical protein
PA3068	*gdhB*	-3.39		NAD-dependent glutamate dehydrogenase
PA3096	*xcpY*		2.79	general secretion pathway protein L
PA3109	*cvpA*	4.53		hypothetical protein
PA3111	*folC*	2.53		folylpolyglutamate synthetase
PA3123		-3.21		conserved hypothetical protein
PA3126	*ibpA*		-2.64	heat-shock protein IbpA
PA3161	*himD*		3.85	integration host factor beta subunit
PA3162	*rpsA*	2.90		30S ribosomal protein S1
PA3167	*serC*	2.62		3-phosphoserine aminotransferase
PA3168	*gyrA*	6.17		DNA gyrase subunit A
PA3171	*ubiG*		-2.87	3-demethylubiquinone-9 3-methyltransferase
PA3173	*yciK*		-3.00	probable short-chain dehydrogenase
PA3178			5.97	hypothetical protein
PA3182	*pgl*	3.24	3.39	6-phosphogluconolactonase
PA3183	*zwf*		2.68	glucose-6-phosphate 1-dehydrogenase
PA3186	*oprB*	-2.96		Glucose/carbohydrate outer membrane porin OprB precursor
PA3188	*gltG*	-2.69		probable permease of ABC sugar transporter
PA3189	*gltF*	-2.99		probable permease of ABC sugar transporter
PA3194	*edd*		3.18	phosphogluconate dehydratase
PA3195	*gapA*		4.44	glyceraldehyde 3-phosphate dehydrogenase
PA3202	*yciI*	4.59	3.56	conserved hypothetical protein
PA3216		-3.04		hypothetical protein
PA3217	*cyaB*		-2.55	CyaB
PA3227	*ppiA*	-3.99	-2.42	peptidyl-prolyl cis-trans isomerase A
PA3229			3.08	hypothetical protein
PA3231			8.27	hypothetical protein
PA3232			2.62	probable nuclease
PA3234	*yjcG*		4.43	probable sodium:solute symporter
PA3235	*yjcH*		4.35	conserved hypothetical protein
PA3245	*minE*		-3.50	cell division topological specificity factor MinE
PA3259			2.42	hypothetical protein
PA3266	*capB*	39.06		cold acclimation protein B
PA3280	*oprO*		-2.75	Pyrophosphate-specific outer membrane porin OprO precursor
PA3285		3.22	2.82	probable sigma-70 factor, ECF subfamily
PA3292			-13.59	hypothetical protein
PA3299	*fadD1*		-2.76	long-chain-fatty-acid--CoA ligase
PA3309	*uspK*	-2.57		conserved hypothetical protein
PA3311		-2.47		conserved hypothetical protein
PA3316			2.40	probable permease of ABC transporter
PA3332			-4.34	conserved hypothetical protein
PA3346		-3.99		probable two-component response regulator
PA3347		-3.86		hypothetical protein

PA3349			2.29	probable chemotaxis protein
PA3351	*flgM*		3.02	FlgM
PA3352		4.08	10.58	hypothetical protein
PA3354			5.62	hypothetical protein
PA3361	*lecB*	6.20		fucose-binding lectin PA-IIL
PA3371			3.07	hypothetical protein
PA3383	*phnD*		-2.70	binding protein component of ABC phosphonate transporter
PA3397	*fpr*	3.88		ferredoxin--NADP+ reductase
PA3415			3.57	probable dihydrolipoamide acetyltransferase
PA3416		-3.62		probable pyruvate dehydrogenase E1 component, beta chain
PA3417			7.94	probable pyruvate dehydrogenase E1 component, alpha subunit
PA3418	*ldh*		11.44	leucine dehydrogenase
PA3431	*ywbG*	5.63	4.36	conserved hypothetical protein
PA3432		7.51	7.91	hypothetical protein
PA3436		3.12		hypothetical protein
PA3438	*folE1*	2.17		GTP cyclohydrolase I precursor
PA3450	*lsfA*	5.81	7.06	probable antioxidant protein
PA3451		2.64	9.46	hypothetical protein
PA3458		-2.32		probable transcriptional regulator
PA3459	*asnB*	-3.59		probable glutamine amidotransferase
PA3461	*yhfE*	-2.46		conserved hypothetical protein
PA3465	*yfiS*	-4.74		conserved hypothetical protein
PA3472		2.28		hypothetical protein
PA3473		2.53		hypothetical protein
PA3479	*rhlA*	4.34		rhamnosyltransferase chain A
PA3489	*rnfA*		-4.38	conserved hypothetical protein
PA3496			-2.71	hypothetical protein
PA3525	*argG*	3.15		argininosuccinate synthase
PA3526	*motY*		3.28	probable outer membrane protein precursor
PA3531	*bfrB*		-4.20	bacterioferritin
PA3533	*ydhD*	4.60	3.45	conserved hypothetical protein
PA3536			5.14	hypothetical protein
PA3567		2.48	2.51	probable oxidoreductase
PA3568	*ymmS*	-16.53		probable acetyl-coa synthetase
PA3569	*mmsB*	-15.16		3-hydroxyisobutyrate dehydrogenase
PA3570	*mmsA*	-6.75		methylmalonate-semialdehyde dehydrogenase
PA3572			4.21	hypothetical protein
PA3576			6.68	hypothetical protein
PA3578		6.67	6.75	conserved hypothetical protein
PA3580	*ybaK*		-3.36	conserved hypothetical protein
PA3584	*glpD*	11.15		glycerol-3-phosphate dehydrogenase
PA3601	*ykgM*	2.25		conserved hypothetical protein
PA3611		2.44		hypothetical protein
PA3618	*ygaD*		5.40	conserved hypothetical protein
PA3621	*fdxA*	3.56		ferredoxin I
PA3622	*rpoS*		5.13	sigma factor RpoS
PA3636	*kdsA*		-2.67	2-dehydro-3-deoxyphosphooctonate aldolase
PA3637	*pyrG*		-2.79	CTP synthase
PA3642	*rnhB*		-2.53	ribonuclease HII
PA3644	*lpxA*		-3.34	UDP-N-acetylglucosamine acyltransferase

PA3645	*fabZ*		-2.91	(3R)-hydroxymyristoyl-[acyl carrier protein] dehydratase
PA3646	*lpxD*		-4.87	UDP-3-O-[3-hydroxylauroyl] glucosamine N-acyltransferase
PA3648			-4.07	probable outer membrane protein precursor
PA3652	*uppS*		-2.68	undecaprenyl pyrophosphate synthetase
PA3653	*frr*		-2.86	ribosome recycling factor
PA3655	*tsf*	2.55		elongation factor Ts
PA3662		-8.00		hypothetical protein
PA3668			3.08	conserved hypothetical protein
PA3674		-3.39		hypothetical protein
PA3684			3.45	hypothetical protein
PA3686	*adk*		-3.17	adenylate kinase
PA3688			15.53	hypothetical protein
PA3691		-2.50		hypothetical protein
PA3692		-2.53		probable outer membrane protein precursor
PA3698		-2.45		hypothetical protein
PA3720		2.68		hypothetical protein
PA3722			-5.04	hypothetical protein
PA3723	*yqjM*	-3.37		probable FMN oxidoreductase
PA3724	*lasB*	-3.83		elastase LasB
PA3737	*dsbC*	2.70		thiol:disulfide interchange protein DsbC
PA3740			4.38	hypothetical protein
PA3741			-3.70	hypothetical protein
PA3742	*rplS*	2.18	-4.78	50S ribosomal protein L19
PA3743	*trmD*	2.86		tRNA (guanine-N1)-methyltransferase
PA3744	*rimM*	2.92		16S rRNA processing protein
PA3745	*rpsP*	3.04		30S ribosomal protein S16
PA3747		3.10		conserved hypothetical protein
PA3754	*yeaB*	3.05	3.85	hypothetical protein
PA3756	*yafK*	3.00		hypothetical protein
PA3770	*guaB*		-2.56	inosine-5'-monophosphate dehydrogenase
PA3784		-6.60		hypothetical protein
PA3785		-7.56		conserved hypothetical protein
PA3796			7.41	hypothetical protein
PA3804			-3.57	hypothetical protein
PA3806	*yfgB*		-3.21	conserved hypothetical protein
PA3808	*yfhJ*	3.16	4.19	conserved hypothetical protein
PA3811	*hscB*	2.97		heat shock protein HscB
PA3812	*iscA*	2.85	2.49	probable iron-binding protein IscA
PA3813	*iscU*	11.05	8.81	probable iron-binding protein IscU
PA3814	*iscS*	5.62	2.78	L-cysteine desulfurase (pyridoxal phosphate-dependent)
PA3815		8.88		conserved hypothetical protein
PA3817		2.37		probable methyltransferase
PA3818	*suhB*	2.65		extragenic suppressor protein SuhB
PA3819	*ycfJ*		3.90	conserved hypothetical protein
PA3822	*yajC*		-2.61	conserved hypothetical protein
PA3824	*queA*		-3.60	S-adenosylmethionine:trna ribosyltransferase-isomerase
PA3833			4.23	hypothetical protein
PA3841	*exoS*	-3.03		exoenzyme S
PA3844			2.34	hypothetical protein

PA3846			7.10	hypothetical protein
PA3847		-3.07		conserved hypothetical protein
PA3848		-2.88		hypothetical protein
PA3851			3.47	hypothetical protein
PA3858	*aapJ*	-8.70		probable amino acid-binding protein
PA3899		2.08	3.69	probable sigma-70 factor, ECF subfamily
PA3903	*prfC*		-2.89	peptide chain release factor 3
PA3905			-4.23	hypothetical protein
PA3906			-3.47	hypothetical protein
PA3907			-2.85	hypothetical protein
PA3908			-5.48	hypothetical protein
PA3914	*moeA1*	2.71	2.68	molybdenum cofactor biosynthetic protein A1
PA3915	*moaB1*	7.01	5.88	molybdopterin biosynthetic protein B1
PA3916	*moaE*	2.70	3.73	molybdopterin converting factor, large subunit
PA3918	*moaC*	4.39	4.47	molybdopterin biosynthetic protein C
PA3922		-3.89		conserved hypothetical protein
PA3940			-2.61	probable DNA binding protein
PA3945		-2.98		conserved hypothetical protein
PA3957			4.42	probable short-chain dehydrogenase
PA3966		2.84	-2.67	hypothetical protein
PA3967			-6.13	hypothetical protein
PA3974	*ladS*		2.41	Lost Adherence Sensor, LadS
PA3979		2.83		hypothetical protein
PA3982		2.25		conserved hypothetical protein
PA3986			59.52	hypothetical protein
PA4002	*rodA*	2.23		rod shape-determining protein
PA4007	*proA*	2.92		gamma-glutamyl phosphate reductase
PA4012			3.96	hypothetical protein
PA4021			3.20	probable transcriptional regulator
PA4026			2.68	probable acetyltransferase
PA4029	*dedA*	3.02	2.49	conserved hypothetical protein
PA4031	*ppa*	4.27		inorganic pyrophosphatase
PA4034	*aqpZ*	6.87	5.59	aquaporin Z
PA4044	*dxs*	3.39		1-deoxyxylulose-5-phosphate synthase
PA4045	*btuF*	2.22		conserved hypothetical protein
PA4046		4.45		hypothetical protein
PA4051	*thiL*	2.50		thiamine monophosphate kinase
PA4067	*oprG*		-2.72	Outer membrane protein OprG precursor
PA4069		2.45		hypothetical protein
PA4079		-2.37		probable dehydrogenase
PA4090		4.92	9.29	hypothetical protein
PA4112		-2.44		probable sensor/response regulator hybrid
PA4129		-2.90	-3.58	hypothetical protein
PA4130			-3.05	probable sulfite or nitrite reductase
PA4131			-5.74	probable iron-sulfur protein
PA4132			-5.18	conserved hypothetical protein
PA4133	*ccoN*	-4.43	-10.55	cytochrome c oxidase subunit (cbb3-type)
PA4134		-4.75	-18.67	hypothetical protein
PA4135			-2.79	probable transcriptional regulator
PA4139		-35.52	-284.87	hypothetical protein
PA4140			-8.17	hypothetical protein
PA4141			-5.68	hypothetical protein
PA4142			-10.00	probable secretion protein

PA4147	*acoR*	7.01	10.16	transcriptional regulator AcoR
PA4175	*piv*		2.76	protease IV
PA4176	*ppiC2*	2.22	3.13	peptidyl-prolyl cis-trans isomerase C2
PA4218		-3.28		probable transporter
PA4219	*yfpB*	-6.77		hypothetical protein
PA4220	*fptB*	-4.16	4.81	hypothetical protein
PA4221	*fptA*	-2.94		Fe(III)-pyochelin outer membrane receptor precursor
PA4222	*pchI*	-3.89		probable ATP-binding component of ABC transporter
PA4223	*pchH*	-6.02		probable ATP-binding component of ABC transporter
PA4224	*pchG*	-8.90		pyochelin biosynthetic protein PchG
PA4225	*pchF*	-4.53		pyochelin synthetase
PA4226	*pchE*	-5.13		dihydroaeruginoic acid synthetase
PA4227	*pchR*	-3.47		transcriptional regulator PchR
PA4228	*pchD*	-6.21		pyochelin biosynthesis protein PchD
PA4229	*pchC*	-8.75		pyochelin biosynthetic protein PchC
PA4230	*pchB*	-2.63		salicylate biosynthesis protein PchB
PA4231	*pchA*	-9.31		salicylate biosynthesis isochorismate synthase
PA4237	*rplQ*		-3.91	50S ribosomal protein L17
PA4239	*rpsD*		-2.53	30S ribosomal protein S4
PA4240	*rpsK*	2.73	-2.45	30S ribosomal protein S11
PA4241	*rpsM*	4.15		30S ribosomal protein S13
PA4245	*rpmD*	2.45		50S ribosomal protein L30
PA4247	*rplR*		-3.46	50S ribosomal protein L18
PA4248	*rplF*	4.52		50S ribosomal protein L6
PA4250	*rpsN*	2.70		30S ribosomal protein S14
PA4252	*rplX*		-3.53	50S ribosomal protein L24
PA4253	*rplN*		-3.05	50S ribosomal protein L14
PA4254	*rpsQ*		-3.42	30S ribosomal protein S17
PA4261	*rplW*		-2.75	50S ribosomal protein L23
PA4262	*rplD*	3.21		50S ribosomal protein L4
PA4263	*rplC*	3.02		50S ribosomal protein L3
PA4264	*rpsJ*	5.85		30S ribosomal protein S10
PA4265	*tufA*	2.54		elongation factor Tu
PA4268	*rpsL*	2.45		30S ribosomal protein S12
PA4270	*rpoB*	2.50		DNA-directed RNA polymerase beta chain
PA4271	*rplL*		-2.95	50S ribosomal protein L7 / L12
PA4272	*rplJ*	4.65		50S ribosomal protein L10
PA4273	*rplA*	3.41		50S ribosomal protein L1
PA4274	*rplK*	5.93		50S ribosomal protein L11
PA4275	*nusG*	10.56		transcription antitermination protein NusG
PA4276	*secE*	9.32		secretion protein SecE
PA4290		-3.34		probable chemotaxis transducer
PA4292		2.23		probable phosphate transporter
PA4293	*pprA*		6.91	two-component sensor PprA
PA4294			18.28	hypothetical protein
PA4296	*pprB*		7.11	two-component response regulator, PprB
PA4297	*tadG*	-10.49		TadG
PA4298		-10.92		hypothetical protein
PA4299	*tadD*	-5.04		TadD
PA4300	*tadC*	-5.81	2.29	TadC
PA4301	*tadB*	-4.48		TadB
PA4302	*tadA*	-17.19		TadA ATPase
PA4303	*tadZ*	-8.11		TadZ

PA4304	*rcpA*	-19.23		RcpA
PA4305	*rcpC*	-4.80	6.54	RcpC
PA4306	*flp*		33.22	Type IVb pilin, Flp
PA4312		-3.61		conserved hypothetical protein
PA4317			-3.49	hypothetical protein
PA4318			-2.98	hypothetical protein
PA4319			-2.66	conserved hypothetical protein
PA4326		2.73	7.50	hypothetical protein
PA4328		-2.55		hypothetical protein
PA4348			-2.31	conserved hypothetical protein
PA4352			4.30	conserved hypothetical protein
PA4362		-2.79		hypothetical protein
PA4377		-4.10		hypothetical protein
PA4379			2.95	conserved hypothetical protein
PA4385	*groEL*		-2.64	GroEL protein
PA4386	*groES*	-2.78	-2.89	GroES protein
PA4405			-5.16	hypothetical protein
PA4407	*ftsZ*	-7.57	-2.81	cell division protein FtsZ
PA4408	*ftsA*	-4.12		cell division protein FtsA
PA4409	*ftsQ*	-3.13	-2.77	cell division protein FtsQ
PA4410	*ddlB*	-3.09		D-alanine--D-alanine ligase
PA4411	*murC*	-3.98		UDP-N-acetylmuramate--alanine ligase
PA4412	*murG*	-2.47		UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase
PA4413	*ftsW*	-2.66		cell division protein FtsW
PA4414	*murD*	-4.05		UDP-N-acetylmuramoylalanine--D-glutamate ligase
PA4415	*mraY*	-4.64	-4.62	phospho-N-acetylmuramoyl-pentapeptide-transferase
PA4416	*murF*	-2.57		UDP-N-acetylmuramoylalanyl-D-glutamyl-2, 6-diaminopimelate--D-alanyl-D-alanyl ligase
PA4417	*murE*	-3.16		UDP-N-acetylmuramoylalanyl-D-glutamate-2, 6-diaminopimelate ligase
PA4418	*ftsI*	-2.80		penicillin-binding protein 3
PA4419	*ftsL*	-7.62	-2.73	cell division protein FtsL
PA4420	*ylxA*	-4.17		conserved hypothetical protein
PA4421	*yabB*	-3.82		conserved hypothetical protein
PA4426	*yraP*	-2.71	-8.66	conserved hypothetical protein
PA4427	*sspB*		-3.04	stringent starvation protein B
PA4428	*sspA*		-5.23	stringent starvation protein A
PA4429			-3.88	probable cytochrome c1 precursor
PA4430			-8.23	probable cytochrome b
PA4431			-4.98	probable iron-sulfur protein
PA4432	*rpsI*	3.82	-3.15	30S ribosomal protein S9
PA4433	*rplM*	2.99	-4.06	50S ribosomal protein L13
PA4442	*cysN*	2.27	3.27	ATP sulfurylase GTP-binding subunit/APS kinase
PA4443	*cysD*	3.94	3.64	ATP sulfurylase small subunit
PA4451	*yrbA*	2.61		conserved hypothetical protein
PA4452		-4.03		conserved hypothetical protein
PA4458	*yrbI*		-3.27	conserved hypothetical protein
PA4459	*yrbK*		-4.34	conserved hypothetical protein
PA4460	*yhbN*		-2.54	conserved hypothetical protein
PA4461	*yhbG*		-2.50	probable ATP-binding component of ABC transporter
PA4463	*yhbH*		2.73	conserved hypothetical protein

PA4467			5.13	hypothetical protein
PA4468	*sodM*	-1.28	3.72	superoxide dismutase
PA4469		-1.88	2.89	hypothetical protein
PA4470	*fumC1*		5.58	fumarate hydratase
PA4474	*tldD*	-2.61		conserved hypothetical protein
PA4479	*mreD*	2.88	-2.46	rod shape-determining protein MreD
PA4480	*mreC*		-3.22	rod shape-determining protein MreC
PA4481	*mreB*		-3.33	rod shape-determining protein MreB
PA4482	*gatC*	3.58		Glu-tRNA(Gln) amidotransferase subunit C
PA4494			-5.12	probable two-component sensor
PA4507		-5.10		hypothetical protein
PA4523			5.68	hypothetical protein
PA4528	*pilD*		-4.21	type 4 prepilin peptidase PilD
PA4530			-2.81	conserved hypothetical protein
PA4542	*clpB*	-3.47	-3.76	ClpB protein
PA4544	*rluD*	2.75		pseudouridine synthase
PA4545	*comL*		-3.28	competence protein ComL
PA4550	*fimU*	4.31		type 4 fimbrial biogenesis protein FimU
PA4551	*pilV*	2.55		type 4 fimbrial biogenesis protein PilV
PA4556	*pilE*	2.25		type 4 fimbrial biogenesis protein PilE
PA4563	*rpsT*	16.23	-3.53	30S ribosomal protein S20
PA4567	*rpmA*	7.25		50S ribosomal protein L27
PA4568	*rplU*	5.20		50S ribosomal protein L21
PA4569	*ispB*		-2.55	octaprenyl-diphosphate synthase
PA4570			5.87	hypothetical protein
PA4572	*fklB*	-3.02	3.71	peptidyl-prolyl cis-trans isomerase FklB
PA4573			11.24	hypothetical protein
PA4575			6.22	hypothetical protein
PA4578			-2.62	hypothetical protein
PA4587	*ccpR*		-2.87	cytochrome c551 peroxidase precursor
PA4590	*pra*	-3.04		protein activator
PA4602	*glyA3*	6.77		serine hydroxymethyltransferase
PA4607		-6.13		hypothetical protein
PA4608			11.53	hypothetical protein
PA4611			3.74	hypothetical protein
PA4614	*mscL*	-2.90		conductance mechanosensitive channel
PA4625			-7.49	hypothetical protein
PA4630		2.95	3.42	hypothetical protein
PA4633			2.62	probable chemotaxis transducer
PA4637		11.93	9.48	hypothetical protein
PA4638			-3.00	hypothetical protein
PA4640	*mqoB*		-3.07	malate:quinone oxidoreductase
PA4641		-3.69		still frameshift hypothetical protein
PA4648		-25.98		hypothetical protein
PA4651		-3.01		probable pili assembly chaperone
PA4657			3.04	hypothetical protein
PA4661	*pagL*	-4.21		Lipid A 3-O-deacylase
PA4666	*hemA*	2.48		glutamyl-tRNA reductase
PA4667		2.75		hypothetical protein
PA4670	*prs*	6.05	-3.68	ribose-phosphate pyrophosphokinase
PA4671	*rplY*	4.41		probable ribosomal protein L25
PA4672	*pth*	3.30	-3.74	peptidyl-tRNA hydrolase
PA4673	*ychF*	3.27		conserved hypothetical protein

PA4678	*rimI*		-2.63	peptide n-acetyltransferase RimI
PA4701			2.30	conserved hypothetical protein
PA4702			5.88	hypothetical protein
PA4703		-2.68		hypothetical protein
PA4713			3.66	hypothetical protein
PA4717			4.50	conserved hypothetical protein
PA4726	*cbrB*	3.72	6.66	two-component response regulator CbrB
PA4733	*acsB*	-2.49		acetyl-coenzyme A synthetase
PA4735		-2.74		hypothetical protein
PA4736		-3.07		hypothetical protein
PA4737		-2.90		hypothetical protein
PA4740	*pnp*	2.36		polyribonucleotide nucleotidyltransferase
PA4741	*rpsO*	2.73		30S ribosomal protein S15
PA4742	*truB*	2.86		tRNA pseudouridine 55 synthase
PA4743	*rbfA*		-5.30	ribosome-binding factor A
PA4745	*nusA*	3.83		N utilization substance protein A
PA4746	*yhbC*	7.78	-2.52	conserved hypothetical protein
PA4747	*secG*	3.29		secretion protein SecG
PA4748	*tpiA*	6.20		triosephosphate isomerase
PA4750	*folP*	3.14		dihydropteroate synthase
PA4751	*ftsH*		2.34	cell division protein FtsH
PA4753	*yhbY*	4.59		conserved hypothetical protein
PA4754		2.57		hypothetical protein
PA4756	*carB*	2.50		carbamoylphosphate synthetase large subunit
PA4757	*yeaS*	3.62		conserved hypothetical protein
PA4758	*carA*	2.57		carbamoyl-phosphate synthase small chain
PA4759	*dapB*		-3.95	dihydrodipicolinate reductase
PA4765	*omlA*		-3.19	Outer membrane lipoprotein OmlA precursor
PA4766	*yfjF*	-2.83		conserved hypothetical protein
PA4767	*yfjG*	-2.86		conserved hypothetical protein
PA4768	*smpB*		-2.65	SmpB protein
PA4780			3.61	conserved hypothetical protein
PA4781			5.91	probable two-component response regulator
PA4782		-2.45		hypothetical protein
PA4787		-3.20		probable transcriptional regulator
PA4793			2.85	hypothetical protein
PA4811	*fdnH*	-4.02		nitrate-inducible formate dehydrogenase, beta subunit
PA4833		-2.21	2.63	conserved hypothetical protein
PA4841		-2.45		conserved hypothetical protein
PA4842		-3.00		hypothetical protein
PA4846	*aroQ1*	3.26		3-dehydroquinate dehydratase
PA4852	*yhdG*	4.59		conserved hypothetical protein
PA4853	*fis*		-2.87	DNA-binding protein Fis
PA4859		2.32	2.43	probable permease of ABC transporter
PA4870	*ybiI*	5.86	15.95	conserved hypothetical protein
PA4874	*psiF*		2.53	conserved hypothetical protein
PA4876	*osmE*		3.18	osmotically inducible lipoprotein OsmE
PA4880		-5.13	-4.56	probable bacterioferritin
PA4881		11.55	14.99	hypothetical protein
PA4907	*ydfG*	3.27	2.63	probable short-chain dehydrogenase
PA4913			2.46	probable binding protein component of ABC transporter
PA4915		-4.04		probable chemotaxis transducer

PA4922	*azu*		-2.70	azurin precursor
PA4925		-3.26		conserved hypothetical protein
PA4929		-10.24		hypothetical protein
PA4932	*rplI*	5.38		50S ribosomal protein L9
PA4933		4.85		hypothetical protein
PA4934	*rpsR*	4.35		30S ribosomal protein S18
PA4935	*rpsF*	2.61	-2.56	30S ribosomal protein S6
PA4939	*hisX*	4.46		conserved hypothetical protein
PA4940	*yjeT*	3.53		conserved hypothetical protein
PA4947	*amiB*	-2.83		N-acetylmuramoyl-L-alanine amidase
PA4962	*ybcI*		-2.85	conserved hypothetical protein
PA4965			-2.48	hypothetical protein
PA4971	*aspP*		4.57	adenosine diphosphate sugar pyrophosphatase
PA4997	*msbA*	2.51		transport protein MsbA
PA5000		-2.47		probable glycosyl transferase
PA5028		2.40	2.54	conserved hypothetical protein
PA5033			-3.24	hypothetical protein
PA5040	*pilQ*	-2.54	-3.81	Type 4 fimbrial biogenesis outer membrane protein PilQ precursor
PA5041	*pilP*	-2.34	-4.56	type 4 fimbrial biogenesis protein PilP
PA5042	*pilO*		-2.96	type 4 fimbrial biogenesis protein PilO
PA5043	*pilN*		-2.63	type 4 fimbrial biogenesis protein PilN
PA5044	*pilM*		-2.61	type 4 fimbrial biogenesis protein PilM
PA5045	*ponA*		-2.71	penicillin-binding protein 1A
PA5047			2.38	hypothetical protein
PA5048		4.09	3.17	probable nuclease
PA5049	*rpmE*	14.73		50S ribosomal protein L31
PA5053	*hslV*		-2.40	heat shock protein HslV
PA5054	*hslU*		-4.68	heat shock protein HslU
PA5055			-3.12	hypothetical protein
PA5058	*phaC2*	-2.86		poly(3-hydroxyalkanoic acid) synthase 2
PA5062			3.15	conserved hypothetical protein
PA5097	*hutT*	-2.61		probable amino acid permease
PA5101		-3.22		hypothetical protein
PA5108		-2.37		hypothetical protein
PA5116			2.48	probable transcriptional regulator
PA5117	*typA*	3.98		regulatory protein TypA
PA5129	*grx*	2.40		glutaredoxin
PA5130	*yibN*		-2.38	conserved hypothetical protein
PA5139			-4.21	hypothetical protein
PA5148	*yggX*	-2.77		conserved hypothetical protein
PA5150			3.92	probable short-chain dehydrogenase
PA5164	*rmlC*	-2.67		dTDP-4-dehydrorhamnose 3,5-epimerase
PA5170	*arcD*		3.53	arginine/ornithine antiporter
PA5171	*arcA*	-3.36		arginine deiminase
PA5172	*arcB*	-6.56		ornithine carbamoyltransferase, catabolic
PA5173	*arcC*	-4.88		carbamate kinase
PA5176	*yrfE*	2.65	3.22	conserved hypothetical protein
PA5182			2.48	hypothetical protein
PA5200	*ompR*		2.43	two-component response regulator OmpR
PA5208		-5.33		conserved hypothetical protein
PA5212			3.29	hypothetical protein
PA5213	*gcvP1*	-4.88		glycine cleavage system protein P1

PA5231	*yhiH*		-2.74	probable ATP-binding/permease fusion ABC transporter
PA5232	*yhiI*	-2.68		conserved hypothetical protein
PA5236	*ubiB*		3.03	probable aromatic hydrocarbon reductase
PA5239	*rho*	5.83		transcription termination factor Rho
PA5240	*trxA*	3.80		thioredoxin
PA5242	*ppk*	-2.44	-3.39	polyphosphate kinase
PA5244	*yohD*	2.22		conserved hypothetical protein
PA5245	*yhbL*	-5.42		conserved hypothetical protein
PA5249		3.67	3.08	hypothetical protein
PA5253	*algP*		4.42	alginate regulatory protein AlgP
PA5255	*algQ*		6.01	Alginate regulatory protein AlgQ
PA5258	*hemX*		2.33	hypothetical protein
PA5259	*hemD*		2.57	uroporphyrinogen-III synthetase
PA5261	*algR*		6.06	alginate biosynthesis regulatory protein AlgR
PA5263	*argH*		-2.46	argininosuccinate lyase
PA5271			2.53	hypothetical protein
PA5274	*rnk*	2.99		nucleoside diphosphate kinase regulator
PA5275	*cyaY*	2.78	9.15	conserved hypothetical protein
PA5276	*lppL*	2.22		Lipopeptide LppL precursor
PA5287	*amtB*	4.44	4.27	ammonium transporter AmtB
PA5288	*glnK*		5.53	nitrogen regulatory protein P-II 2
PA5295		2.51		hypothetical protein
PA5298	*xpt*	6.75		xanthine phosphoribosyltransferase
PA5302	*dadX*		-2.41	catabolic alanine racemase
PA5303		-3.11	-4.65	conserved hypothetical protein
PA5315	*rpmG*	10.78		50S ribosomal protein L33
PA5316	*rpmB*	5.89		50S ribosomal protein L28
PA5331	*pyrE*	4.12		orotate phosphoribosyltransferase
PA5333			-2.48	conserved hypothetical protein
PA5337	*rpoZ*	5.97	4.41	RNA polymerase omega subunit
PA5338	*spoT*	2.94		guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase
PA5340		3.66		hypothetical protein
PA5346	*sadB*		2.80	SadB
PA5348		-3.29	2.67	probable DNA-binding protein
PA5359		-2.59	2.56	hypothetical protein
PA5366	*pstB*		-4.34	ATP-binding component of ABC phosphate transporter
PA5367	*pstA*		-2.99	membrane protein component of ABC phosphate transporter
PA5373	*betB*	2.37		betaine aldehyde dehydrogenase
PA5374	*betI*	13.48	3.45	transcriptional regulator BetI
PA5380	*gbdR*		6.30	GbdR
PA5383	*yeiH*	6.44	7.63	conserved hypothetical protein
PA5408			3.57	hypothetical protein
PA5409			3.68	hypothetical protein
PA5424	*yeaQ*		2.49	conserved hypothetical protein
PA5435	*oadA*		-3.78	probable transcarboxylase subunit
PA5438			2.63	probable transcriptional regulator
PA5446		4.25		hypothetical protein
PA5460		10.48	24.15	hypothetical protein
PA5468		3.62	3.84	probable citrate transporter
PA5479	*gltP*		-5.25	proton-glutamate symporter
PA5481			3.69	hypothetical protein

PA5482			5.68	hypothetical protein
PA5490	*cc4*		-5.85	cytochrome c4 precursor
PA5494			3.43	hypothetical protein
PA5504		4.80		probable permease of ABC transporter
PA5505			-3.63	probable TonB-dependent receptor
PA5527			12.39	hypothetical protein
PA5528			3.08	hypothetical protein
PA5531	*tonB*		4.56	TonB protein
PA5543		-3.14		hypothetical protein
PA5546		-3.35		conserved hypothetical protein
PA5553	*atpC*		-7.97	ATP synthase epsilon chain
PA5554	*atpD*		-3.60	ATP synthase beta chain
PA5555	*atpG*		-6.51	ATP synthase gamma chain
PA5556	*atpA*		-3.86	ATP synthase alpha chain
PA5557	*atpH*		-3.87	ATP synthase delta chain
PA5558	*atpF*		-5.16	ATP synthase B chain
PA5559	*atpE*	-3.08	-5.31	atp synthase C chain
PA5560	*atpB*		-7.12	ATP synthase A chain
PA5568	*yidC*	2.58	-3.96	conserved hypothetical protein
AF035937cds10	*wbpT*		-3.27	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF035937cds11	*wbpU*		-2.44	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF035937cds3	*wzz*		-2.68	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF035937cds4	*wbpO*		-4.26	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF035937cds7	*wzx*		-2.62	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF035937cds8	*wbpR*		-3.14	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF035937cds9	*wbpS*		-3.12	*P. aeruginosa* IATS gene cluster for O-antigen biosynthesis
AF043558cds			-1.54	*P. aeruginosa* beta-lactamase OXA-13-1
L81176cds4	*fliS*		2.47	*P. aeruginosa* flagellin
M21652cds		8.97	4.05	*P. aeruginosa* P1 type IV pilin precursor
Pae_tRNA_Asn		1.82		tRNA_Asparagine
Pae_tRNA_Gln		2.35		tRNA_Glutamine
Pae_tRNA_Gly		2.83		tRNA_Glycine
Pae_tRNA_His		2.83		tRNA_Histidine
Pae_tRNA_Ile		3.26		tRNA_Isoleucine
Pae_tRNA_Leu		4.17		tRNA_Leucine
Pae_tRNA_Lys		4.94		tRNA_Lysine
Pae_tRNA_Phe		5.25		tRNA_Phenylalanine
Pae_tRNA_Pro		5.61		tRNA_Proline
Pae_tRNA_Ser		8.24		tRNA_Serine
Pae_tRNA_Trp		9.19		tRNA_Tryptophan
Pae_tRNA_Tyr		9.96	2.57	tRNA_Tyrosine
Pae_tRNA_Val			18.38	tRNA_Valine

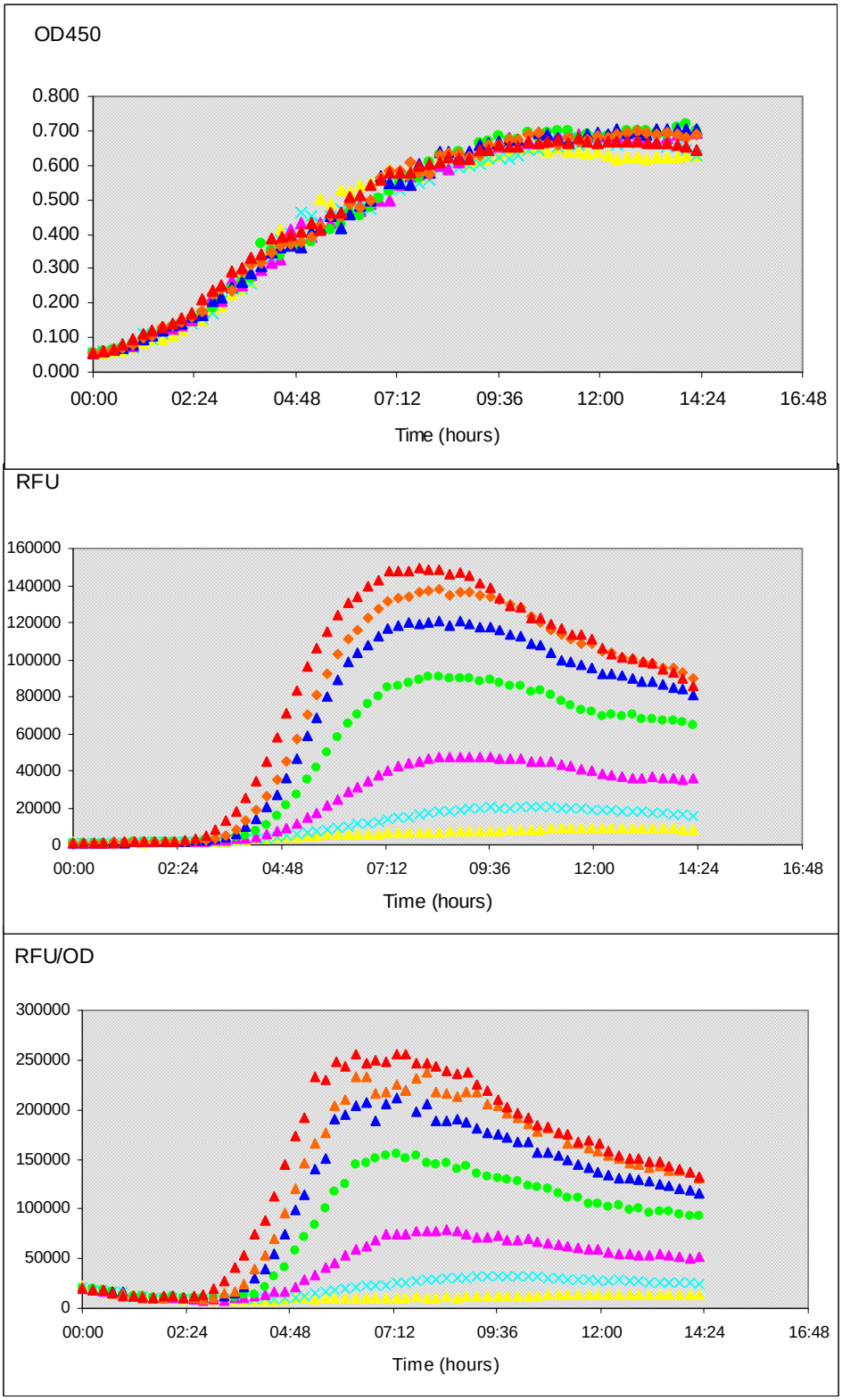

Figure 8-1: OD, Relative Fluorescence Units (RFU) and RFU/OD. Monitor strain PAO1 harbouring the *lasB-gfp*(ASV) fusion. Concentration of protoanemonin: ▲ 174 µM, × 86 µM, ▲ 43 µM, ● 22 µM, ▲11 µM, ♦ 5 µM, ▲ Reference, no compound added. Experiment performed by Mette Elena Skindersoe at the DTU Biocetrum, Denmark.

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